

P.G. JEPPSSON

STUDIES ON
THE BLOOD-BRAIN BARRIER
IN HYPOTHERMIA

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An experimental investigation in the rabbit with special
reference to the effect of intracarotid injection of iodopyracet on the
passage of trypan blue into the brain parenchyma

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MED. LIC., LD

LUND 1962
HÅKAN OHLSSONS BOKTRYCKERI



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BY

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CONTENTS

Introduction	7
The concept of blood-brain barrier.	7
The use of trypan blue in studies on blood-brain barrier	8
The purpose of the present investigation	11

Chapter I

General methods	12
Technique in blood-brain barrier experiments.	12
Cooling technique	15
Solutions	15
Temperature recording	16
Blood pressure measurements.	16
Substance used for producing injury to blood-brain barrier . .	16
Photographic procedure	17
Histologic procedure	17
Statistical methods	18

Chapter II

Assessment of injury to blood-brain barrier	20
Principles for rating the degree of injury on sections	20
Principles for rating the degree of injury on colour photographs	21
Comparison of experimental series	22
Principles for assessment of injury on microscopic examination	22

Chapter III

Control of technique	24
A. Injection of saline	26
I. Body temperature 38° C	26
Injection into common carotid artery	26
Results	27
Injection into common carotid artery with ligation of external	
carotid artery	28
Results	28
II. Body temperature 25° C	28
Injection into common carotid artery.	28
Results	29

III. Body temperature 20° C	29
Injection into common carotid artery	29
Results	30
Injection into common carotid artery with ligation of external carotid artery	30
Results	31
B. Infusion of trypan blue without rinsing of vessels	31
I. Body temperature 38° C	31
Results	32
II. Body temperature 25° C	32
Results	33
III. Body temperature 20° C	33
Results	33
C. Infusion of trypan blue and rinsing with formalin	34
I. Body temperature 38° C	34
Results	34
II. Body temperature 20° C	35
Results	35
D. Injection of air in saline	36
I. Body temperature 38° C	36
Injection into common carotid artery	36
Results	36
Injection into common carotid artery with ligation of external carotid artery	37
Results	37
II. Body temperature 20° C	38
Injection into common carotid artery with ligation of external carotid artery	38
Results	38
E. Infusion of trypan blue and rinsing with saline with air in the tubing . .	39
Results	39
Discussion	40
Summary	46

Chapter IV

Areas in the brain normally permeable to trypan blue	47
Methods	49
Results	50
Body temperature 38° C	50
Body temperature 20° C	50
Discussion	52
Summary	55

Chapter V

Injury to blood-brain barrier produced by Umbradil in normothermia . . .	56
A. Injection into common carotid artery	61
I. Umbradil 50 % of 38° C	61
Results	61

II. Umbradil 50 % of room temperature	63
Application time 20 seconds	63
Results	63
Application time 10 seconds	68
Results	69
Application time 5 seconds	70
Results	70
B. Injection into common carotid artery with ligation of external carotid artery	72
I. Umbradil 50 % of 38° C	72
Application time 40 seconds	72
Results	72
Application time 20 seconds	74
Results	74
C. Injection of Umbradil 50 % with regard to experiments performed in hypothermia	75
I. Injection into common carotid artery after reduction in blood pressure by means of hexamethonium bromide	76
Results	76
II. Injection of cooled Umbradil 50 % into common carotid artery	78
Results	78
D. Comparison of series	79
Discussion	88
Summary	98

Chapter VI

Injury to blood-brain barrier produced by Umbradil in hypothermia	99
A. Body temperature 25° C	106
I. Injection of Umbradil 50 % into common carotid artery	106
Ordinary cooling	106
Results	106
Prolonged cooling-injection time	107
1. Prolonged cooling by delay between end of cooling and injection	107
Results	108
2. Slow cooling	109
Results	109
B. Body temperature 20° C	110
I. Injection into common carotid artery	110
Umbradil 70 % of 38° C	110
Results	110
Umbradil 50 % of room temperature	112
1. Application time 20 seconds	112
Results	112
2. Application time 15 seconds	113
Results	114
Umbradil 50 % of 38° C	115
Results	115
Umbradil 50 % of room temperature. Animals treated with low molecular dextran during cooling	116

Methods	117
Results	118
Treatment with low molecular dextran 6 %	118
Treatment with low molecular dextran 10 %	119
Trypan blue duration about 10 minutes	119
Trypan blue duration 20—30 minutes	120
Trypan blue duration about 60 minutes	121
Trypan blue duration 105—120 minutes	122
Assessment of conjunctival bulbar circulation	123
The effect of noradrenaline on blood pressure in hypothermia and the influence of increased blood pressure at the injection on injury to blood-brain barrier	124
1. Infusion of noradrenaline in saline and in trypan blue solution and measurements of brain and rectal temperature	124
Results	125
2. Injection of Umbradil 50 % of room temperature	126
Results	126
II. Injection into common carotid artery with ligation of external carotid artery	129
Umbradil 70 % of 38° C	129
Results	130
Umbradil 50 % of room temperature	131
1. Application time 40 seconds	131
Results	131
2. Application time 20 seconds	132
Results	133
C. Comparison of series in hypothermia	134
D. Comparison of series with injection of Umbradil in hypothermia and normothermia	143
Discussion	148
Summary	160

Chapter VII

The mode of action of Umbradil in provoking injury to blood-brain barrier.	
The anatomic locus for the restriction of passage of trypan blue into brain parenchyma. A discussion	161
Summary	169
General summary	170
Acknowledgements	171
Tables	173
References	216
Plates	

Abbreviations

Blood-brain barrier	BBB
Trypan blue	trpb
Physiological saline	saline

INTRODUCTION

The concept of blood-brain barrier

In 1885 EHRlich (pp. 67 ff.) observed that a certain dye, coerulein, did not enter the brain but could pass into other organs. LEWANDOWSKY (1900) found that sodium ferrocyanide was toxic when administered into the cerebrospinal fluid but not when given into the blood. BOUFFARD (1906) showed that trypan blue (trpb) did not stain the brain but could stain other organs. GOLDMANN (1909, 1913) confirmed these findings with trpb and showed that the choroid plexuses were stained blue. However, the brain showed staining by trpb after injection into the subarachnoid space. GOLDMANN considered that the choroid plexuses were an obstacle to the passage of toxic substances into the cerebrospinal fluid. STERN (1921) found that certain substances did not pass into the cerebrospinal fluid but that others did. The results were interpreted as evidence of an existing mechanism separating blood and cerebrospinal fluid, and the term "barrière hématoencéphalique" was therefore suggested. STERN pointed out that the term did not indicate a certain structure or function.

GOLDMANN and STERN were of the opinion that substances were transported from blood to brain via the cerebrospinal fluid. This opinion was refuted by WALTER (1930, 1934), who considered it necessary to distinguish between the passage of substances from blood into the brain parenchyma and that into the cerebrospinal fluid and furthermore between cerebrospinal fluid and brain parenchyma. He distinguished between blood-brain barrier, blood-cerebrospinal fluid barrier and cerebrospinal fluid-brain barrier, respectively. The evidence for such a differentiation was mainly the result of studies on dyes which were performed during the preceding decades by various workers and whose results were exhaustively summarized by SPATZ (1934).

In essence it was shown that acid dye stuffs of a so-called semicolloidal type did not pass into the brain parenchyma. With decreasing molecular weight acid dye stuffs were found to pass first into the cerebrospinal fluid and then into the brain parenchyma. Basic dye stuffs, however, passed into the brain parenchyma but not into the cerebrospinal fluid.

With the aid of radioactive isotopes it has been shown during the last two decades that a number of small ions pass from blood to brain paren-

chyma, but only slowly and to a lesser degree than in other organs. Such substances as are lipid soluble, however, are an exception (see BAKAY, 1956). It is now generally accepted that a selective passage from blood to brain parenchyma exists.

In order to explain this phenomenon a number of hypotheses have been put forward. Attention has thus been paid to special properties of the capillary wall in the brain (SPATZ, 1934; KROGH, 1946; BROMAN, 1949; CRONE, 1961), the existence of a glial membrane outside the capillary wall (GÄRTNER, 1927; SCHALTENBRAND & BAILEY, 1928), the existence of a ground substance (HESS, 1955), small or virtually absent extracellular space in the brain (LUMSDEN, 1955; SCHULTZ, MAYNARD & PEASE, 1957; LUSE, 1960b; HAGER, 1961), the electric charge of the substance about to pass (FRIEDEMANN, 1942), the lipid solubility of the substance (KROGH, 1946; MAYER, MAICKEL & BRODIE, 1959), or the existence of an active transport mechanism as suggested by LUMSDEN (1955). CRONE (1961) demonstrated the existence of a mechanism facilitating the passage of glucose from blood into the brain parenchyma. Recently, the selective passage from blood to brain parenchyma has been considered "a reflection of, rather than a limiting factor in" the metabolism of the brain (DOBBING, 1961).

In recent years the term 'blood-brain barrier' (BBB) has been applied to indicate the selective passage of substances from blood to brain, irrespective of prevailing hypotheses (BAKAY, 1956; HERLIN, 1956). For the sake of clarity the term as used in the present work indicates that trpb does not pass into the brain parenchyma in visible amounts in normal conditions. Damage or injury to the blood-brain barrier means that trpb has passed into the brain parenchyma.

The use of trypan blue in studies on blood-brain barrier

As trpb does not normally pass into the brain parenchyma in observable amounts, this substance has been widely used for the study of an abnormally increased passage from blood to brain parenchyma. To begin with, trpb was usually given over a period of several days in fairly small amounts (MACCURDY & EVANS, 1912; MACKLIN & MACKLIN, 1920; MCCLELLAN & GOODPASTURE, 1923; MENDEL, 1928 and others). The location of the trpb was determined with histologic technique.

The use of trpb in short-term experiments has mainly been developed by BROMAN (1938, 1940a, b, c, 1941a, b, 1949), who used large amounts of trpb intravenously and studied the location of the trpb in frozen sections after *post mortem* perfusion of the brain vessels with saline.

DAVSON (1956), referring to RAWSON (1943), says that many dye-

stuffs such as Evans blue and trpb are almost completely adsorbed to the plasma proteins. According to DAVSON the appearance of this type of strongly adsorbed dye-stuff in the ocular or cerebrospinal fluid indicates a break-down of a barrier to protein, which has very little to do with the possible behaviour of the dye-stuff in the free state. DOBBING (1961), referring to GREGERSEN & RAWSON (1943), says that the most important objection to studies with dyes on the selective passage from blood to brain, is the extent to which many dyes become bound, under different circumstances, to plasma proteins. This protein binding is, according to DOBBING, enough to account for the failure of a corresponding proportion of the circulating dye to enter the brain.

In view of the above-mentioned discussion it seems necessary to review the literature concerning the binding of trpb to plasma proteins in order to ascertain to what extent trpb is bound to the plasma proteins in the amounts used in the present investigation.

RAWSON (1943) found that trpb was entirely bound to the serum albumin in a concentration less than 0.004 %. She also pointed out that this was not the case when the concentration of the dye exceeded the binding capacity of the albumin. Using different techniques, RAWSON (1943) and ALLEN & ORAHOVATS (1950) found the maximal binding capacity of albumin to be 2 moles of trpb per mole of albumin. ALLEN & ORAHOVATS (1950) found that trpb occurred in both a free and a bound state in the presence of albumin in concentrations less than 2 moles of trpb per mole of albumin. As the free and the bound portion of a substance are in equilibrium (see KLOTZ, 1953), this means that if the free portion disappears from the compartment, bound molecules are dissociated to maintain the equilibrium. GREGERSEN & RAWSON (1943) showed that the disappearance rate for trpb after intravenous injection was faster than that for Evans blue, which in its turn has been shown to behave like albumin in the blood during the first hours after an injection (SCHULTZ, HAMMARSTEN, HELLER & EBERT, 1953). This means that the passage of trpb from blood into a tissue cannot be taken as evidence of passage of albumin.

In the present experimental conditions the concentration of trpb in plasma far exceeds the maximal binding capacity of albumin as reported in the literature. After filtration, the infused trpb solution contains about 450 mg trpb (molecular weight 960) in 100 ml (see chapter I). If the plasma volume of the animal is supposed to be 100 ml and the albumin (molecular weight about 70 000) concentration 4.4 g/100 ml, the mixed plasma and trpb solution contains almost 7 moles trpb per mole albumin. It is not known whether more trpb might be bound to the albumin in the case of such an excess, but it would be reasonable

to assume that such a binding would be much looser than that of the first 2 moles. RAWSON (1943) found that Evans blue in a concentration exceeding the binding capacity of albumin was bound to the α - and β -globulins. Such an investigation has not been reported in the case of trpb. Even if part of the trpb is bound by these globulins, it must be borne in mind that their concentration is only 1 g/100 ml and that a possible binding of trpb is probably even looser than to albumin.

It seems safe to conclude that the part of trpb which is bound can dissociate from the albumin and that in the present experimental conditions a fair amount of the trpb is in a free state or loosely bound to proteins.

Proof that trpb passes into the brain parenchyma is obtained by microscopic examination. As pointed out by e.g. DAVSON (1956) and DOBBING (1961) the method is rather unsensitive since a certain amount of dye might be present although the concentration is not enough to make it visible. Even if this be so, it does not mean that trpb cannot be used in studies of the BBB, since the penetration of appreciable amounts of trpb indicates that a change has occurred in the hypothetical barriers between blood and brain parenchyma. An attempt to obtain further information by the use of quantitative determination of trpb in BBB experiments is mentioned by BROMAN, EDSTRÖM & STEINWALL (1961), who found that only slightly greater precision is obtained than by mere estimation of the dye intensity on the cut surface of the brain.

The fact that in normal conditions trpb does not pass into the brain parenchyma in visible amounts has not been explained. In addition to the theories mentioned above, the fact that trpb does not stain the brain parenchyma has been considered due to a lack of affinity of the brain for trpb (KING, 1938a, b). He found that trpb stained only connective tissue in the body. However, trpb stains the brain if administered into the cerebrospinal fluid (GOLDMANN, 1913; SPATZ, 1934). BROMAN (1940b) repeated KING's experiments and found that epithelial cells too were stained except in the brain, when large doses of trpb were used. In view of this it does not seem possible to agree with KING that the lack of connective tissue explains the fact that trpb does not pass into the brain parenchyma.

Microscopic studies of the structures stained by the dye made it possible to draw conclusions with regard to the locus of the BBB (BROMAN, 1949, 1955; BROMAN, EDSTRÖM & STEINWALL, 1961). This does not seem to be possible with such methods as autoradiography according to BAKAY (1956) and LEE & OLSZEWSKI (1960), who found that the resolution of x-ray film emulsions did not admit of a precise localization of the radioactivity in relation to the components of the vessel wall or perivascular tissue.

The purpose of the present investigation

In recent years much attention has been paid to the brain in hypothermia. The experimental work done has mainly centred on the effect of anoxia, while such an important factor as the selective passage from blood to brain parenchyma in hypothermia has received less attention.

In a preliminary study on the rabbit JEPPSSON & NIELSEN (1955) found that brains showing injury to the BBB produced by the intracarotid injection of the roentgen contrast medium Umbradil (iodopyracet) became less frequent as the body temperature fell owing to cooling in cold air. The present investigation was undertaken in order to ascertain whether the hypothermic state of the animal interferes with the flow of the contrast medium through the vessels of the brain or the passage of trpb into the brain parenchyma in such a way that the absence of injury to the BBB can thereby be explained.

GENERAL METHODS

Technique in blood-brain barrier experiments

220 rabbits with a mean body weight of $2.1 \text{ kg} \pm 0.4$ (S.D.) were used for the experiments. Most of the animals were male but also female rabbits were used. The animals were anesthetized with Nembutal (Abbot), about 40—50 mg/kg body weight given into an ear vein.

The experiments were mainly performed according to the technique described by BROMAN & OLSSON (1948, 1956a).

The rabbit was placed in the supine position during the experiment. Tracheotomy was performed and a Y-formed glass cannula was inserted into the trachea and connected with a breathing pump inflating it with air at intermittent positive pressure. The respiration rate was kept at about 30—40 resp./min. The stroke volume of the breathing pump was regulated in such a way that the movements of the animal's chest were definite but not vigorous. With this kind of artificial respiration the animals were kept well oxygenated at normal body temperature as judged by the colour of the arterial blood and the blood pressure.

A burr hole 1 cm in diameter was made with a trephine on the left side of the skull in the parietal region. The dura was as a rule left intact.

For the infusion of the trpb solution a glass cannula was inserted into the femoral vein and connected with a saline container. The saline was allowed to drip slowly into the vein.

The mean arterial blood pressure was recorded by means of a glass cannula inserted into the femoral artery and connected with a mercury manometer by a column of saline. The manometer was so placed that both columns of mercury were on a level with the heart in the zero position. The glass cannula was filled with a 5 per cent solution of heparin (Vitrum, Stockholm, Sweden). In many of the experiments the blood pressure was also recorded on a kymograph.

The external jugular veins were then ligated and clamps put on the cranial end of the veins. The vein was cut between the ligature and the clamp.

The common carotid artery on the left side was ligated caudally and a small clamp applied cranially. An incision was made between the ligature and the clamp. A straight edged steel cannula with an inner diameter of 1.0—1.1 mm and a length of 60 mm was connected with a mercury manometer by means of a rubber tube and an Y-formed glass

cannula. The free end of the glass cannula was connected with a rubber tube to a syringe. The injection system was first filled with saline, special care being taken to avoid air bubbles. The injection system was flooded with saline when the steel cannula was inserted into the common carotid artery. Then the steel cannula was fixed with a ligature and the clamp applied cranially on the common carotid artery was removed. The blood then flowed into the injection system which was again carefully examined for air bubbles. The blood was then driven back into the artery and a clamp placed on the rubber tube near the steel cannula. The volume of the injection system with the exception of the tube connected to the mercury manometer was about 2 ml.

A pilot injection was then made with saline to check if the blood was expelled from the cerebral blood vessels as inspected through the burr hole in the skull. If there was no expulsion of blood the position of the cannula was corrected and a new pilot injection performed. Occasionally the injection had to be performed on the other (right) side.

The rubber tube connected with the steel cannula was clamped when the syringe with the experimental solution was put in its place.

Before the injection of the experimental solution the return flow of blood into the injection system was checked and the blood driven back again. Then the clamps on the jugular veins were removed and the blood was allowed to flow out this way.

During the injection the animal's head had to be turned either to the left or the right in order to make possible inspection of the pial vessels. In some experimental series the animal's head was regularly turned to the left. Otherwise the position of the head was not noted.

The injection pressure was checked on the mercury manometer and kept at about 200 mm Hg. The time of the injection was recorded on a stop watch by an assistant who also inspected the pial vessels (the vessels on the surface of the brain) and registered the time (with an accuracy of 0.5 seconds) in which the blood was expelled from these vessels during the injection. With BROMAN & OLSSON (1949) this time was called application time. The degree of expulsion of the blood from the pial vessels during the injection was also noted with regard to whether blood in arteries and veins or in both was expelled and whether the expulsion of the blood was constant or only intermittent in these vessels.

In some of the experiments the time for the refilling of the cerebral blood vessels with blood after the end of the injection was also recorded. When the decided application time was up, the injection was ended. Then the rubber tube connected to the steel cannula in the common carotid artery and the cranial parts of the jugular veins were clamped. The injected amount of solution was recorded.

After the completion of the intracarotid injection about 100 ml of the trpb solution was infused through the femoral vein. The animals of normal body temperature were killed 15 minutes after the beginning of the infusion of the trpb solution (trpb duration). In hypothermic animals the trpb duration was meant to be 30 minutes but it happened that the animals died earlier. *Post mortem* perfusion of saline was performed either at death or 30 minutes after the beginning of the trpb infusion. In some experiments the trpb duration was increased up to 2 hours.

The animals were killed by opening the chest and cutting the right ventricle of the heart.

The vessels were rinsed in the following way: A cannula was inserted into the thoracic part of the aorta, care being taken to avoid air, and by means of a column of saline and a T-formed glass cannula the aortic cannula was connected with two bottles, one containing saline and the other 10 per cent formalin. The tube connected to the bottle with formalin was clamped. After infusion of 300 ml of saline the clamp was applied over the tube to the bottle with saline and thus formalin in about 200 ml was infused. The saline was always infused first.

Then the brain was removed and the normally blue-stained areas (choroid plexuses, median eminence, hypophysis, epiphysis and area postrema, when not otherwise stated) of the brain checked and the extent of a possible staining of the brain noted, as was the staining of the dura mater on the base of the skull.

In the experiments on normothermic animals, which lasted about 2 hours, the body temperature was maintained by electric heating of the operating table. Body temperature, respiration rate, pulse and blood pressure and room temperature were checked 3—4 times during the course of these experiments.

In some series the external carotid artery was ligated before the injection of the damaging solution into the common carotid artery. Such a modification in this kind of experiment was used by BLOOR, WRENN & MARGOLIS (1951, 1954) and STEINWALL (1958). In the present experiment the internal carotid artery was identified as the first vessel to arise from the dorsal aspect of the common carotid artery (JEPPSSON & OLIN, 1960). The ligature was then applied on the external carotid artery and by injection of saline the expulsion of the blood from the pial vessels was checked.

Care was taken not to damage the innervation of the carotid sinus and the superior cervical ganglion. No dissection was made in order to check whether the occipital artery originated anomalously from the internal carotid artery.

In these experiments, too, the external jugular veins were cut. This means that the amount of recirculating contrast medium was reduced since blood from the brain is drained mainly through the external jugular veins (JEPPSSON & OLIN, 1960). Also in other details the experiments were pursued as described above.

Cooling technique

The hypothermic state was induced by surface cooling (VIRTUE, 1955; COOPER & ROSS, 1960 and McMILLAN & MACHELL, 1961).

The animals were anesthetized, tracheotomized, and artificially respired as described above. The wound in the neck was sutured. Artificial respiration is necessary in hypothermia for maintaining respiration when barbiturates are used as an anesthetic agent (VIRTUE, 1955). According to SWAN, ZEAVIN, HOLMES & MONTGOMERY (1953) and OSBORN (1953) hyperventilation is necessary for optimal cardiac activity. The respiration parameters were the same as for normothermic animals and kept up during the whole experiment. This means that the hypothermic animals were hyperventilated. The animals were sprayed with tap water at a temperature of about 8–12° C. The temperature of the animals and of the water and the pulse rate were recorded every 30 minutes, in some experiments every 15 minutes.

When the body temperature was 1–2° C above the decided level, the cooling in water was stopped and the animal was placed on the operating table. As a rule the body temperature continued to fall, as described among others by VIRTUE (1955), COOPER & ROSS (1960). If this did not occur the animal was covered with a towel soaked in cold water.

Then the BBB experiments were performed as described above.

Solutions

The trpb solution was freshly prepared according to BROMAN (1949). 500 mg trypan blue (Merck, Darmstadt, Germany) was solved in 100 ml boiling saline, which was allowed to cool down to room temperature and then filtered through a Jena G 2 glass filter. In order to ascertain the order of magnitude of the remains of trpb on the filter in the present experimental conditions, 3 dried filters were weighed in separate experiments. The amount of trpb remaining on the filters was found to be 39, 42 and 46 mg, respectively. The trpb solution less easily gives micro-embolism if the trpb is solved in saline rather than in distilled water (BROMAN, 1949). In experiments on animals of normal body temperature the trpb solution and the saline used for injection were heated to 38° C in a water thermostat. In experiments on hypothermic animals the so-

lutions were of room temperature. The temperature of such a trpb solution was measured in 63 experiments and found to be $21.7^{\circ}\text{C} \pm 1.3$ (S.D.). For the corresponding saline solution the temperature was $20.2^{\circ}\text{C} \pm 0.8$ (S.D.) as measured in 82 experiments.

Temperature recording

The room temperature was recorded on a mercurial thermometer (50° , 1/10) suspended from the roof in the warmest part of the laboratory. The mean room temperature in 220 experiments was $22.0^{\circ}\text{C} \pm 0.7$ (S.D.).

The rectal temperature was recorded on an electric thermometer (type TE 3, Elektrolaboriet, Copenhagen, Denmark) operating according to the thermo-couple principle with an accuracy of $\pm 0.1^{\circ}\text{C}$, provided with a standard scale, range $16\text{--}46^{\circ}\text{C}$, and with a rectal applicator (type RK 2). This thermometer was inserted into the rectum 9 cm above the anus. According to LEE (1939) the temperature in the rectum of the rabbit is constant 7.5 cm above the anus. In a few experiments a mercurial thermometer was used. The limits of normal body temperature of any group of rabbits will be from 38.4 to 41.1°C according to LEE.

The brain temperature was measured with an applicator type K 8, 0.5 mm in diameter and 30 mm long, inserted through the burr hole into the brain down to just above the base of the skull. This applicator was also used for measuring the temperature of the solutions. The electric thermometer was supplied with 2 plug-sockets and readings from the 2 applicators were possible with a minimum delay by means of a change-over switch. The electric thermometer was regularly checked in a water thermostat.

The temperature of the cooling water was measured on a mercurial thermometer (32° , 1/10) and read with an accuracy of 0.5°C .

Blood pressure measurements

The levels of the mean arterial blood pressure are given with an accuracy of 5 mm Hg. Values between 0 and 10 mm Hg are presented as <10 mm Hg. The blood pressure rise during the injection was measured from the centre of the tracing to the top of the peak, the pre-injection level being used as a base.

Substance used for producing injury to blood-brain barrier

In the present investigation a roentgen contrast medium, iodopyracet (Umbradil, manufactured by Astra, Södertälje, Sweden) was used for

producing the injury to the BBB. According to the manufacturer this is a water soluble diethanolamine salt 3,5-diiodo-4-pyridone-N-acetic acid with a molecular weight of 510.10. The salt contains 49.8 % iodide (STRAIN, ROGOFF & MAURITZEN, 1961). The contrast medium is furnished in sterile solutions in ampoules (20 ml) in concentrations of 35, 50 and 70 %. The viscosity at different temperatures was determined by the manufacturer on a Höppler viscosimeter. The viscosity of Umbradil 50 % at 37° C was 2.20 centipoise (cp) and at 20° C 3.56 cp. Umbradil 70 % yielded a value of 4.19 cp at 37° C. It was impossible to determine the viscosity at 20° C for Umbradil 70 % as the solution crystallized in the viscosimeter at that temperature.

At 25° C the specific gravity for Umbradil was 50 %—1.27; 70 %—1.38.

As a rule the contrast medium was of room temperature when injected. The temperature of Umbradil 50 % as measured in 51 experiments was $21.3^{\circ}\text{C} \pm 1.3$ (S.D.). In some experiments it was heated in a water thermostat to 38° C and drawn up into a heated syringe. Umbradil 70 % was always heated to 38° C as otherwise it precipitated on being drawn up in the syringe.

In table 1 are listed the commercial, generic and chemical names of the roentgen contrast media referred to in the present work.

Photographic procedure

Colour photographs were taken of most of the brains after removal and before sectioning. The camera used was an Exacta Varex Camera (manufactured by Ihagee, Dresden, Germany) with a Tessar lens (1:4.5, $f=10.5$ cm) placed on a bellows. The films were Kodachrome type A for artificial light (K 135 A). Two photoflood lamps (Philips, type PF 308E/21, 500 W) were used as light source. The brains were placed on a glass plate. The distance from the film to the object table was 45—47 cm. Lens opening 22. The exposure time was varied with the aid of an exposure meter and according to the bluishness of the brain. The films were developed at the Hasselblad laboratories, Spånga, Sweden.

Histologic procedure

After removal the brains were put in 10 per cent formalin and on the following day cut in the frontal plane in frozen sections. As a rule sections 500 μ thick were cut, 3 sections being made from 4 parts of the brain, the frontal, parietal and the occipital parts, and the cerebellum with the pons or the medulla oblongata. As a rule the side orientation of the brain section was marked on the slides. This was done in the following

Table 1. *Commercial, generic and chemical names of roentgen contrast media referred to in the present work. From STRAIN, ROGOFF & MAURITZEN (1961).*

Commercial name	Generic name (Pharmacopeia of the United States)	Chemical composition
Umbradil Diodrast Nycotrast Dijodon	Iodopyracet	Diethanolamine salt of 3.5-diiodo-4-pyridone-N- acetic acid
Umbradil forte		40.5 % w/v Diethanolamine Salt and 9.5 % w/v Diet- hylamine Salt of 3.5-diiodo- 4-pyridone-N-acetic acid
Umbradil-methyl- glucamine		Methylglucamine salt of 3.5-diiodo-4-pyridone-N- acetic acid
Triurol Urokon	Sodium Acetrizoate	Sodium salt of 3-acet- amido-2.4.6-triiodo- benzoic acid
Hypaque	Diatrizoate Sodium	Sodium salt of 3.5- diacet-amido-2.4.6- triiodobenzoic acid
Miokon	Sodium Diprotrizoate	Sodium salt of 3.5- dipropionamido-2.4.6- triiodobenzoic acid
Urografin Renografín	Sodium and Methyl- glucamine salts of Diatrizoate	

way: The parts were always cut from behind and the section remained on the knife before mounting in glycerine. The remainder of the brain was kept in formalin and it was thus possible to check side marking, which could also be controlled by the colour photographs. In some experiments the brains were completely sectioned and in some only 3—4 coronal sections were cut through the parietal region of the brain.

Statistical methods

In the statistical treatment of the data the arithmetic mean, the standard deviation (S.D.), standard error of the mean (S.E.) and the *t*-test were calculated as described by DAVIES (1957).

The following non-parametric statistical methods were adopted from SIEGEL (1956): The Fisher exact probability test for 2×2 contingency tables, the Mann-Whitney U test, the Friedman two-way analysis of variance and the Kruskal-Wallis one-way analysis of variance. In the Mann-Whitney U test the ranking starts with the lowest score.

The significance of the values obtained by the various tests was determined by the tables for the P values for the respective tests provided by SIEGEL (1956). The value of P was doubled in two-tailed tests.

A test is called one-tailed when the difference between the null hypothesis and the alternative hypothesis as regards direction is stated. When no prediction of the direction can be made in advance the test is called two-tailed. Thus if it is not possible to presume before an experiment whether a difference, if any, from a control experiment will take a negative or a positive direction, the test is two-tailed. If on the other hand it is possible to presume before the experiment that a difference, if any, from a control experiment can take only one direction, the test is one-tailed.

When P was less than 0.05 the difference was regarded as probable (almost significant). When P was less than 0.01 the difference was regarded as significant.

ASSESSMENT OF INJURY TO BLOOD-BRAIN BARRIER

The degree of staining can be presumed roughly to correspond to the degree of the injury to the BBB. Stasis with no staining of the parenchyma was considered a more pronounced damage than the most intense staining. The area with stasis had a red colour on macroscopic examination.

Both colour photographs and sections of the brain were rated according to the following scale.

++-+	stasis
++++	intense staining
++(+)	
++	definite staining
+(+)	
+	slight staining
(+)	scattered blue streaks or dots with a tendency to confluence
(+?)	scattered blue streaks or dots
neg.	no staining

The rating was done after most of the experiments had been carried out.

If only the olfactory bulbs or single areas smaller than the olfactory bulb were stained the brain was considered negative. Exceptional staining occurring beneath the burr hole was regarded as an artefact owing to the trephination procedure. The character and extent of such small lesions, however, is given in the reports of the results.

As the material divides up into 2 large groups, one with a high-grade injury and a second with a low-grade injury or none at all, the former consisting almost only of brains from the series with injection of Umbradil at normal body temperature and the latter of experiments performed in hypothermia and of technique controls, no attempt was made to rate under unbiased conditions.

Principles for rating the degree of injury on sections

The sections were arranged in experimental series. Each experiment was individually assessed. The non-sectioned parts of the brain were also rated and, when necessary, cut macroscopically in thick slices. By

this procedure it was checked whether a bleaching of the mounted sections had occurred or not.

The degree of injury was rated separately in the following areas: Cerebral cortex, hippocampus, white matter (corpus callosum, centrum ovale, internal capsule and anterior commissure), basal nuclei (caudate nucleus, putamen and globus pallidus), hypothalamus, thalamus, quadrigeminal bodies, cerebellum, pons and medulla oblongata. The character of the staining was also noted i.e. patchy, streaky or dotted. Then the prevailing degree of injury for the whole brain was assessed on all the sections, mounted and non-mounted. In the case of a variation in the degree of injury to the whole brain, the maximal lesion, with an extent compatible with that of half the cerebellum, was taken as representative of the whole brain.

The supply areas of the internal carotid arteries and the vertebral arteries on both sides were ranked with regard to the degree and extent of the injury on the sections. Of the structures listed above, those from the cerebral cortex up to and including the hypothalamus are mainly supplied from the internal carotid artery. The rest, from the thalamus, is mainly supplied from the vertebral arteries (JEPPSSON & OLIN, 1960). Damage to the parasagittal area (supplied by the anterior cerebral artery) on the side opposite to the injection side with no lesion within the rest of the area of the internal carotid artery on that side, was accounted for on the area of the internal carotid artery on the side of the injection.

Principles for rating the degree of injury on colour photographs

The degree of injury on the brain surface was assessed on colour photographs according to the above-mentioned scale. The variation in exposure time was corrected for in the following way: 4 strips from brains with different degree of staining rated as +, +(), ++ and +++ were photographed under the same conditions as the original brains with varying exposure times. At the rating, a photograph of these strips with the actual exposure time was used.

When rated, the brains were arranged in experimental series. Each brain was assessed separately. The degree of injury to the cerebral and cerebellar hemispheres was particularly noted and the most stained of these was taken as representative of the brain. Both the upper and nether surface of the brain were assessed. The examination of the colour photographs was made on the same horisontal light cabinet with the same light source. As the emulsions of the films were not the same, the results of the rating of the colour photographs were not used for comparison of series. The results of the rating serve to demonstrate the difference, if

any, between the brain surface and the cut surface. It was found on macroscopic examination that the differences obtained agreed with the appearance of the brains.

Comparison of experimental series

Two experimental series were compared with regard to the results from the above-mentioned assessment on sections. The series were also compared directly, i.e. the sections from the actual 2 series were examined simultaneously and the brains were ranked according to the degree and extent of the lesion. The significance of the results was examined with the Mann-Whitney U test. Comparison of the normothermic and hypothermic Umbradil-series was performed by 2 judges. One of these was the author.

Principles for assessment of injury on microscopic examination

The sections were examined microscopically in transmitted and in reflected light (dissecting microscope). A slight diffuse staining of the parenchyma seemed to be easier to detect on examination in reflected light.

In the present work diffuse staining means a uniformly stained area, small or large, in which the outlines of the cells or the nuclei are not visible. Staining of the parenchymal nuclei and staining of the endothelial nuclei means that the trpb has accumulated uniformly within the nuclei to such an extent that these are clearly visible (plate III:1).

Uniform staining of the cell nuclei in vital staining is regarded as a severe damage to the cell (GROSS, 1911; ROST, 1911; MACCURDY & EVANS, 1912; MCCLELLAN & GOODPASTURE, 1923). HÖBER (1909) stated that diffuse staining of the nuclei by acid dye stuffs could be due to a toxic action of the dye. According to MÖLLENDORFF (1920) it is doubtful if uniform staining always means the death of the cell. He considered the possibility that such a staining of the nucleus may be a sign of increased amounts of the dye being available to the cell. For a review on vital staining the reader is referred to SPATZ (1934).

An area was denoted as the site of stasis if several vessels contained packed red corpuscles. As this was seen after the *post mortem* saline perfusion of the vessels, it was taken as a sign of true stasis (ROTNES & KREYBERG, 1932).

The sections were made 500 μ thick according to GRÖNTOFT (1954). It was found that diffuse staining was not visible on microscopic examination in sections less than 200 μ thick. Staining of the parenchymal nuclei was visible in sections 20 μ thick.

In order to test in which dilutions the original 0.5 per cent trpb solution was visible on microscopic examination a chamber with a depth of 0.5 mm was made. After instillation of the diluted trpb solutions, the chamber was covered with a micro cover glass. It was found that the dilutions at which the trpb solution appeared as blue were as follows: Macroscopic examination 2 %; reflected light, magn. $\times 40$ 4 %; transmitted light, magn. $\times 20$ 5 %; magn. $\times 80$ and $\times 320$ 8 %. This means that a trpb concentration of about 0.02 per cent will be visible on microscopic examination in reflected light.

In the survey of the microscopic examination in the column 'parenchymal staining' in the tables, brains with a few small areas of parenchymal staining are presented as neg., while pos. denotes that more than a few small areas were stained. Virtual absence of staining of the intracerebral vessels was denoted as neg. and presence of such staining in more than a few vessels as pos.

For the anatomy of the rabbit's brain the reader is referred to WINKLER & POTTER (1911). The anatomic nomenclature used by RANSON & CLARK (1951) was followed as closely as possible. Exceptions are e.g. the pyriform lobe (the rabbit's rhinencephalon, WINKLER & POTTER, 1911); the terms 'dorsal and ventral hippocampus', denote the 2 separated, rostral parts of the hippocampus (mainly consisting of the cornu Ammonis); the term 'convexity of cortex', denotes the part of the cerebral cortex located between the parasagittal and the rhinal cortex.

Intracerebral vessels with a diameter of about $10\ \mu$ or less were regarded as capillaries. The others were denoted as 'larger vessels'.

CONTROL OF TECHNIQUE

The sources of error of the technique for BBB experiments have been discussed by BROMAN (1949) and in certain respects by BROMAN & OLSSON (1948, 1956a) and SMITH, CAUDILL, MOORE, PEYTON & FRENCH (1951).

SMITH *et al.* (1951) pointed out that the force of the injection of the damaging agent into the carotid artery might damage the vessel walls owing to increased intraluminal pressure. In their experiment they therefore used a low injection pressure. BROMAN & OLSSON (1956a) found that a high injection pressure could be injurious in combination with Umbradil 35 % in innocuous doses. In view of this the injection pressure was kept on the same level in the present investigation and the size of the injection system was the same in all experiments. This will also increase the chances for the injected volumes to be of about the same size.

Precipitated Umbradil in the tip of the syringe might be a cause of embolism (BROMAN & OLSSON, 1956a). For this reason the Umbradil solution was not drawn up into the syringe until immediately before the injection. For the same reason the steel cannula inserted into the common carotid artery was well rinsed after use and was checked by rinsing with saline before the insertion into the artery.

The possibility of clot formation in the common carotid artery was also discussed by BROMAN & OLSSON (1956a). To minimize this risk the common carotid artery was only clamped during the insertion of the steel cannula into the artery. The time lag between insertion and injection of Umbradil was as short as possible. The return of blood into the injection system was checked before the intracarotid injection and taken as an indication of free passage into the artery.

The stasis due to clamping of the jugular veins does not give any BBB lesion (BROMAN & OLSSON, 1948).

BROMAN (1949) and BROMAN & OLSSON (1956a) considered artificial respiration to be a source of error as it might possibly give air embolism due to rupture of the lungs. As artificial respiration was used in the present investigation it was thought necessary to check the technique on this point.

The method of rinsing the vessels with saline at the end of the experiment in studies of the BBB with vital dyes has been used by WISLOCKI & KING (1936) and SKOOG (1937) in order to obtain a better view of the

normally stained or the damaged areas of the brain. BROMAN (1937, 1938, 1940a, 1941b) rinsed the vessels in order to avoid *post mortem* diffusion of the vital dye and staining of undamaged parts of the brain in experiments with bromphenol blue. BROMAN & OLSSON (1948) and BROMAN (1949) rinsed the vessels so that the residual blue colour of the brain was due only to the penetration of the trpb into the brain parenchyma. This might also render microscopic examination unnecessary when the brain is undamaged.

BROMAN (1940b) perfused the abdominal organs with saline after infusion of trpb and found that the vessels of the liver and the kidney were still stained with trpb, as was the parenchyma. The brain that in the same animal was rinsed too, never showed any staining of the vessel wall or the parenchyma. TSCHIRGI (1952) thought that this difference might be explained by assuming diffusion of the vital dye from the parenchyma of the liver back into the walls of the vessels of the liver during the rinsing of the vessels. BROMAN, EDSTRÖM & STEINWALL (1961) considered that rinsing of the cerebral vessels with saline increased rather than diminished the accuracy in determining extravasation of trpb.

BROMAN (1950) found that rinsing with formalin of an otherwise normal animal, supravitaly infused with trpb, caused an artefactual staining of the brain. On the basis of these experiments and his own findings on supravitaly stained human and rabbit foetus, GRÖNTOFT (1954) attributed a diffuse staining of the parenchyma, without or with only scanty staining of the parenchymal nuclei and the endothelial cell nuclei around a vessel filled with trpb, to a *post mortem* breakdown of the BBB and to formalin fixation.

BROMAN (1938) studied air embolism on guinea pigs with bromphenol blue as indicator of the injury to the BBB and found a diffuse or patchy macroscopic appearance of the lesion. BROMAN (1940a) performed similar experiments with different kinds of emboli, also air in a 5 per cent gum arabic-tyrode solution. It was given to guinea-pigs and cats, in the former animals about 0.1—0.2 ml and in the latter somewhat more. The solution was injected into the common carotid artery by a fine needle or through a branch to this artery. Air administered in this way was found to give a passage to trpb into the brain parenchyma immediately after the injection of the emboli containing solution. The wall was stained down into the capillaries and the passage of trpb as a rule took place around these vessels. ROZDILSKY & OLSZEWSKI (1957) injected 0.3 ml air 3 times at 30 minutes' interval in cats pretreated with radioactive iodinated bovine albumin. On autoradiography patchy lesions were found in the brains. LEE & OLSZEWSKI (1959 b) injected air into the common carotid artery of cats and rabbits. The dose was 0.2 ml given twice

at 5 minutes' interval. Radioactive iodinated bovine albumin was used as an indicator of lesion with the help of autoradiography. In 3 rabbits trpb was used. In cats the radioactivity was maximal the first hour and then quickly declined. In rabbits air embolism failed to provoke a lesion detectable by radioactive albumin. In experiments with trpb as an indicator of the lesion, the hemisphere on the injected side was tinged blue. It was confirmed that the lesion was transient. No microscopic studies were performed in the trpb experiments.

Thus the review of the literature gives the impression that a species difference might exist in the effect of air injected into the carotid artery for rabbits and cats, as pointed out by LEE & OLSZEWSKI (1959 b). As rabbits were used in the present experiments and the reported experiments on this animal are few and not performed with microscopic examination, further studies on the effect of intracarotid injection of air on the BBB seemed necessary.

As pointed out by BROMAN (1949) it seems possible that air which has entered the rinsing system might give an artefactual staining of the brain in experiments on the BBB. However, no experiment intended to demonstrate this has been reported. It is known that air that enters the brain vessels *in vivo* can give a lesion of the BBB and it is not unreasonable to assume that such an artefactual staining might also occur during the rinsing *post mortem*, but it is of interest to obtain information concerning the amount of air necessary for provoking such lesions.

In the present chapter, experiments are presented referring to:

1. The used technique with injection of saline instead of Umbradil under the actual experimental conditions.
2. Such possible errors in the technique as a) the effect of defective rinsing, b) the effect of infusion of formalin without preceding rinsing with saline, c) the evaluation of the quantities of air on injection into the vessels of the brain of the rabbit, necessary for giving an impairment of the BBB under the actual experimental conditions, and d) the effect of air in the rinsing system.

A. INJECTION OF SALINE

I. BODY TEMPERATURE 38°C

Injection into common carotid artery

Experimental. — In 4 animals saline of room temperature was injected into the left common carotid artery. The application time was intended to be 20 seconds. In 5 animals the temperature of the injected saline was 38°C. Four of the brains were dissected for the purpose of exam-

ining the intercolumnar tubercle. Five of the brains were cut in sections. Four of these were completely cut. One was only cut with 4—6 sections from the parietal and occipital part.

Results

The data of the experiments are given in table 33 and 34. The blood pressure was as a rule about 60—80 mm Hg at the beginning of the experiment but dropped to about 30—40 mm Hg towards the end of the experiment.

The injection pressure as a rule fell at the end of the injection and it was difficult to keep it constant. The intended application time of 20 seconds was only achieved in 3 out of the 9 experiments. In one experiment no application time was achieved. In C 96 and C 100 a herniation of the brain occurred after the injection of the saline.

When the clamps on the jugular veins were removed the blood pressure fell 10—40 mm Hg. During the injection of the saline a slight increase in the blood pressure took place, amounting to the pre-injection level and even 5—10 mm Hg above for a few seconds. During the trpb infusion the blood pressure again increased and fell when the infusion was finished. One of the animals died during the trpb infusion.

Macroscopic examination. — The areas normally permeable to trpb were stained as was the dura mater, except in C 95, in which this was faintly blue. The intercolumnar tubercle was stained in one out of 4 brains examined in this respect. The 2 brains with herniations were stained on the surface of the herniated part but only one of them, C 98, in the parenchyma beneath the herniation. Otherwise the brain parenchyma was unstained.

Microscopic examination. — In none of the 5 histologically investigated brains (C 94, C 96, B 96, C 99 and C 98) was any staining of the walls of the pericallosal arteries observed.

The intracerebral vessels, i.e. the vessels within the cerebral parenchyma, were not stained in the walls with the exception of vessels that probably run to the choroid plexuses. In 3 of the brains trpb was found in the lumen of the capillaries, particularly in the cortex. A few red blood corpuscles were found in some vessels. In C 94 one vessel in the cortex was found to be stained in the walls. The lumen was filled with red blood corpuscles.

In C 98 the herniated part of the left cortex showed a small hemorrhage and staining of the wall of vessels and surrounding brain parenchyma. Otherwise no blue staining of the brain parenchyma occurred in the histologically examined brains with the exception of the areas normally permeable to trpb.

Injection into common carotid artery with ligation of external carotid artery

As an application time of 20 seconds in the experimental series with injections of saline into the common carotid artery was not always obtained, it was thought necessary to perform the experiments also after ligation of the external carotid artery so as to get evidence of the possible effect of the anoxia induced by the injection. It would also mean a control of the technique in this kind of experiments.

Experimental. — In 4 animals saline of 38° C was injected into the left common carotid artery after ligation of the external carotid artery. The application time was 40 seconds in 3 of the animals and 30 seconds in one animal. The brain was cut, 3 sections being made from 4 parts of the brain.

Results

The data of the experiments are given in table 35. In 2 of the experiments the injection pressure was increased to expel the blood in the pial vessels. The intended application time was always achieved. The time from the end of the injection to the return of the blood to the pial vessels varied from 2 to 4 seconds. The blood pressure fell 10 to 20 mm Hg on removal of the jugular clamps. During the injection the blood pressure rose about 30 mm Hg in 2 of the experiments. In the others the rise was less than 10 mm Hg. The injected volume was fairly large in one experiment.

Macroscopic examination. — The areas normally permeable to trpb including the supraoptic crest were stained blue. The dura mater was blue bilaterally, in C 263 more on the right side. All brains were unstained with the exception of C 173, which was stained a faint greyish blue on the surface.

Microscopic examination. — In none of the brains were the pericallosal arteries stained. The capillaries of the cortex were often filled with trpb, particularly in C 173. There was no positive evidence of staining of the walls of the vessels or of the brain parenchyma.

II. BODY TEMPERATURE 25°C

Injection into common carotid artery

Experimental. — Five animals were cooled. Saline of room temperature was then injected into the left common carotid artery with an application time of 20 seconds. In 3 of the animals the respiration rate was increased in order to provoke air embolism. The intention was to kill the animals 30 minutes after the beginning of the trpb infusion. No

colour photographs were taken. Only one brain was sectioned with 3 coronal sections through the parietal region.

Results

The data of the experiments are given in tables 36 and 37. The cooling time in water varied from 30 to 55 minutes. The rectal temperature at the injection into the common carotid artery varied from 23.8° C to 25.0° C. The body temperature dropped about 1° C during the experiment. In 3 animals the brain temperature was measured before the injection and agreed well with the rectal temperature measured at the same time.

The blood pressure was low in 3 of the animals. The pulse rate was about a third of the values at normal body temperature. The injected volume of saline was about 40 ml. The intention was to kill the animals 30 minutes after the beginning of the trpb infusion. This was only achieved in 2 out of 5 animals. The other 3 died after the completion of the trpb infusion. The blood pressure at the end of the experiments was very low.

Macroscopic examination. — The choroid plexuses and the median eminence were stained blue in all experiments. The area postrema was stained in the 3 experiments examined. On inspection of the brains after removal C 31, C 32 and C 33 showed a few fine, pin-prick dots in the parietal region on the left side, which may be due to the trephination. In C 32 the pia mater was stained a faint blue. When it was stripped off the underlying hemisphere was not stained. On the cut surface, C 29 and C 32 showed a staining along the track of the needle for the measurement of the brain temperature. In C 33 no such staining was noticed. In all brains the brain parenchyma was not stained with the above-mentioned exceptions.

Microscopic examination. — The 3 sections from C 32 showed no trpb in the lumen of the vessels nor in the brain parenchyma. The choroid plexuses were stained blue in the vessel walls.

III. BODY TEMPERATURE 20° C

Injection into common carotid artery

Experimental. — Seven animals were cooled. Saline of room temperature was injected into the left common carotid artery with an application time of 20 seconds. The intention was to kill the animals 30 minutes after the beginning of the trpb infusion.

C 60 and C 63 were sectioned with 2—3 sections through the parietal region. The other 5 brains were cut, 3 sections being made from 4 parts of the brain.

Results

The data of the experiments are given in tables 38 and 39. The cooling in water was finished at a body temperature of about 22°C . The cooling time in water varied from 45 to 100 minutes. The rectal temperature at the injection of the saline varied from 21.6 to 20.2°C . During the experiment the body temperature as a rule rose about 0.5°C .

The decided application time was achieved.

The blood pressure was about 30 mm Hg at the time of injection into the common carotid artery. It did not fall at the removal of the jugular clamps and rose at most 10 mm Hg during the injection. The pulse rate was about 50/min.

The injected amount of saline was generally about 40 ml. The time for the return of blood in the pial vessels after the end of the injection varied from 3 to 7 seconds.

Only 2 out of the 7 animals lived the desired 30 minutes. In one of the animals the heart stopped before the trpb infusion was completed. Four of the animals lived varying lengths of time after the completion of the trpb infusion. During the later part the blood pressure as a rule fell gradually during or after the completion of the trpb infusion.

Macroscopic examination. — None of the brains was stained, either on the surface or on sections, except the areas normally permeable to trpb. The staining of the dura mater was faint in C 60 and C 290 and patchy in C 63. In C 274 the dura mater was stained blue on both sides. In the others the staining was more pronounced on the right side.

Microscopic examination. — None of the brains was stained in the parenchyma. In some of the brains trpb occurred in the capillaries and sometimes also in the larger vessels. This was specially marked in the cortex. No obvious difference between the two hemispheres was noted in this respect. It has to be stressed that these remnants of trpb in the vessels were small and that as a rule there was an almost complete rinsing of the vessels. The pericallosal arteries were not stained in the walls. In C 276 two vessels were found in the fore part of the brain near the lateral ventricle which were diffusely stained in the walls. The vessels had no remnants of trpb in the lumen and were fairly large.

Injection into common carotid artery with ligation of external carotid artery

Experimental. — Four animals were cooled. After ligation of the left external carotid artery the saline was injected into the left common carotid artery with application times up to 150 seconds. To obtain such long application times it was necessary to change syringes during the injection.

tion. The brain was sectioned, 3 sections being made from 4 parts of the brain.

Results

The experimental data are given in tables 40 and 41. The cooling time in water varied from 52 to 75 minutes. The rectal temperature at the injection was about 20.5°C . The injected amount of saline consisted of 2 groups of magnitude, one about 140 ml and the other about 60 ml. The time for the return of the blood from the pial vessels after the end of the injection was very long, up to 42 seconds. Only in one experiment was it fairly short, 5 seconds. This animal had a higher blood pressure during the experiment than the others, in which the blood pressure was about 20 mm Hg. Three of the animals lived about 30 minutes after the beginning of the trpb infusion.

As a rule the blood pressure did not change during the injection procedure but in C 264 a rise of about 35 mm Hg took place during the injection and lasted for about 4 minutes. In one animal no pulse was recordable before the injection, but was 32/min. afterwards.

Macroscopic examination. — The areas normally permeable to trpb were stained with the exception of C 260 (the animal with the very low blood pressure), in which only the choroid plexuses were stained. The dura mater was not stained in C 260 and was faint in C 262 and C 266. The staining was more pronounced on the right side in C 264. Neither on the surface nor on the sections was the brain parenchyma stained in any of the experiments.

Microscopic examination. — None of the brains was stained in the parenchyma. The vessels of the parenchyma were as a rule well rinsed even if trpb sometimes remained in the vessels, usually in the capillaries of the cortex. The wall of the pericallosal arteries was not stained.

B. INFUSION OF TRYPAN BLUE WITHOUT RINSING OF VESSELS

I. BODY TEMPERATURE 38°C

Experimental. — Three animals were prepared as in BBB experiments. Saline of room temperature was injected into the left common carotid artery. The animal's head was turned to the left during the injection. The application time was intended to be 20 seconds.

The brain was removed without rinsing or fixation *in situ* and was then put into formalin. The brain was cut, 3 sections being made from 4 parts of the brain. The liver and kidney were also removed for microscopic examination.

Results

The experimental data are given in table 42. The decided application time was never achieved. A slight increase in blood pressure took place during the injection of saline. The blood pressure rose in the beginning of the trpb infusion and then as a rule fell.

Macroscopic examination. — The areas normally permeable to trpb including the supraoptic crest were stained. The dura mater was blue bilaterally. The vessels were blue on the surface as on the cut surface. The brain parenchyma appeared tinged greyish blue.

Microscopic examination. — In none of the brains did any staining occur in the parenchyma of the brain with the exception of one (C 317). In this animal a small area of diffusely blue stained parenchyma was seen in the cerebellum. As a rule it was not possible to say with certainty that the wall of the intracerebral vessels was stained. Fairly large vessels were seen to be definitely stained in the wall as a rare exception. No definite staining of the capillary walls was seen.

Occasionally diffuse staining of the wall of the pericallosal arteries was seen. As a rule, this appeared as a staining of the internal elastic membrane. These vessels were empty in the lumen. Vascular areas with no trpb in the lumen of the vessels were seen. The brain from C 317 was studied in paraffin embedded sections stained with hematoxylin and eosin for the occurrence of granulomatous encephalitis, which was not found.

Liver and kidney. — Macroscopically both organs were blue. The staining of the kidney was most pronounced in the medulla. In the kidney the wall of the larger vessels, the glomeruli and above all the epithelium of the tubuli were stained, occasionally with staining of the nuclei. The liver showed staining of the walls of arteries and veins. The epithelium of the bile ducts was often stained. Occasionally diffuse staining of the liver cells occurred and staining of the nuclei of the liver cells was also seen.

II. BODY TEMPERATURE 25°C

Experimental. — Two rabbits were cooled to about 24° C and then prepared with a burr hole and a cannula in the femoral vein. Trpb was infused and the animals were killed about 25 minutes after the beginning of the trpb infusion. The filling of the pial vessels was observed by means of a corneal microscope. The blood pressure was not recorded. No colour photographs were taken. After removal the brain was cut, 3 sections being made from 3 parts of the brain (prosencephalon, quadrigeminal bodies, cerebellum).

Results

The experimental data are given in tables 43 and 44. The body temperature during the infusion ranged from 23 to 25° C. At the end of the infusion the pial vessels were filled with trpb. The heart was beating at the end of the experiment. The pial vessels were all filled with trpb at the end of the infusion.

Macroscopic examination. — Both brains were stained along the vessels on the surface. On the surface the staining consisted of blue streaks and dots.

Microscopic examination. — No staining of the parenchyma occurred in any of the two brains. The walls of the vessels of the parenchyma were not stained with the exception of one larger vessel, which was stained in one of the brains. In both brains the extracerebral vessels were empty and in one of them the vessel wall was diffusely stained. No staining of the nuclei of the endothelial cells was seen. Vascular areas occurred without trpb in the lumen of the vessels.

III. BODY TEMPERATURE 20° C

Experimental. — Three animals were cooled to about 20° C. Saline of room temperature was injected into the left common carotid artery with an application time of 20 seconds. The animal's head was turned to the left during the injection. The brain was removed 30 minutes after the beginning of the trpb infusion and cut, 3 sections being made from 4 parts of the brain. The liver and kidney were removed for microscopic examination.

Results

The experimental data are given in tables 45 and 46. The cooling time in water was about 60 minutes. The temperature at the injection was about 20.5 °C. The intended application time was achieved. The blood in the pial vessels returned 3 to 6 seconds after the end of the injection. The blood pressure before the injection varied from 25 to 60 mm Hg and only minor changes occurred during the injection procedure. Two of the animals died after the completion of the trpb infusion. The blood pressure as a rule rose during the infusion but fell during the later part, the pulse also decreasing.

Macroscopic examination. — The areas normally permeable to trpb were stained. The dura mater was blue, in C 324 and C 326 mostly on the right side. The brains were blue along the vessels and the surface of the brain appeared faintly greyish blue, as was the cut surface.

In C 324 a more intensely stained spot, 1 mm in diameter, was seen in the right part of the septum pellucidum. In C 326 the left basal part of the quadrigeminal bodies was stained a faintly diffuse blue.

Microscopic examination. — The staining of the walls of the pericallosal arteries was evident in 2 of the brains with staining of the internal elastic membrane. In the third brain it was faint. The lumen of the arteries was empty. The wall of a few of the intracerebral vessels larger than capillaries appeared stained and the lumen was sometimes empty. No staining of the nuclei of the endothelial cells was observed. In C 324 the stained area of the right septum pellucidum consisted of staining of parenchymal nuclei around a vessel filled with trpb and red blood corpuscles. The vessel wall was stained. In C 326 the staining in the left basal part of the quadrigeminal bodies consisted of scattered areas of diffuse parenchymal staining of smaller vessels filled with trpb. The surface appeared rifted. In the cerebellum and the medulla oblongata a thin brim of trpb was found around 3 larger vessels. The filling of the vascular tree with trpb was good and was rather more extensive than in the comparable brains in the series at normal body temperature. The brains from C 324 and C 326 were examined in paraffin embedded sections stained with hematoxylin and eosin and showed no granulo-matous encephalitis.

Liver and kidney. — Macroscopically both organs were blue. In the kidney the blue staining was more pronounced in the cortex than in the medulla.

Microscopically the staining had the same appearance as in the series at normal body temperature. In the kidney some exceptions occurred as the staining of the epithelium of the tubuli seemed less pronounced than at normal body temperature and the glomeruli appeared to be stained more frequently and more pronouncedly than at normal body temperature.

C. INFUSION OF TRYPAN BLUE AND RINSING WITH FORMALIN

I. BODY TEMPERATURE 38°C

Experimental. — Saline of room temperature was injected into the left common carotid artery in 3 animals. The animal's head was turned to the left during the injection. Trpb was infused and the animals killed 15 minutes after the beginning of the trpb infusion. The brain was cut, 3 sections being made from 4 parts of the brain.

Results

The experimental data are given in table 47. The body temperature was on the whole unchanged throughout the experiment. The decided

application time was not achieved. The blood pressure was about 100 mm Hg before the injection and fell 10 mm Hg at most at the removal of the jugular clamps. The blood pressure rise during the injection was 20 mm Hg at most. The blood pressure rose slightly during the trpb infusion.

Macroscopic examination. — The brains were not stained with the exception of the areas normally permeable to trpb. The dura mater was stained blue bilaterally. The rinsing appeared defective bilaterally within the area of the middle cerebral artery in C 322.

Microscopic examination. — None of the brains was stained in the parenchyma. The wall of the intracerebral vessels was stained on exceptional occasions. Some vessels and capillaries contained trpb in the lumen, particularly bilaterally in the cortex of the animal which seemed macroscopically to be defectively rinsed. The wall of the pericallosal arteries appeared faintly stained.

II. BODY TEMPERATURE 20°C

Experimental. — Three rabbits were cooled. Saline of room temperature was injected into the left common carotid artery with an application time of 20 seconds. The animal's head was turned to the left during the injection. The brain was removed 30 minutes after the beginning of the trpb infusion after rinsing with 200 ml formalin. The brain was cut, 3 sections being made from 4 parts of the brain.

Results

The experimental data are given in tables 48 and 49. The cooling time in water varied from 65 to 85 minutes. The body temperature at the injection was about 20.5° C. The decided application time was achieved. The blood returned to the pial vessels 2.5 to 5.5 seconds after the end of the injection. The blood pressure varied from 30 to 70 mm Hg at the injection and was virtually unchanged during the injection procedure. During the first part of the trpb infusion the blood pressure rose and started to fall towards the end of the infusion. The fall continued after the infusion and the pulse rate decreased. The animals died after the end of the trpb infusion.

Macroscopic examination. — The areas normally permeable to trpb were stained. The dura mater was faintly blue or unstained in C 328 and C 329. In C 327 it was stained on both sides. The brain parenchyma was not stained but in C 328 the convexity of the right hemisphere and the cerebellum appeared greyish blue tinged.

Microscopic examination. — The wall of the pericallosal arteries was not stained. Occasionally the intracerebral vessels could be seen stained in the wall when the vessels were rinsed. No staining of the parenchyma occurred. The rinsing of the vessels was poor in the cortex of C 327 and C 328, particularly in the right cortex, the quadrigeminal bodies and the cerebellum. In C 327 the ependyma of the third ventricle was stained faintly and diffusely blue.

D. INJECTION OF AIR IN SALINE

I. BODY TEMPERATURE 38°C

Injection into common carotid artery

Experimental. — In 5 animals saline of room temperature and air were injected into the common carotid artery. Before the injection, measured amounts of air were inserted in increasing amounts by means of a tuberculin syringe into the tubing of the cannula to the left common carotid artery. Air bubbles in the syringe were not removed. The application time was 20 seconds. The brains were completely sectioned with the exception of C 312 and C 313 which were cut, 3 sections being made from 4 parts of the brain.

Results

The data of the experiments are given in table 50. The injected amounts of saline varied from 40 to 60 ml. The amount of air in the tubing of the cannula inserted in the common carotid artery was only a small air bubble (about 0.02 ml air) in C 101, and then increased up to 1—2 ml, which filled the whole tubing up to the Y-tube. The predetermined application time of 20 seconds was only achieved in 2 experiments. The blood pressure was about 70 mm Hg before the injection and fell from 0 to 20 mm Hg. at the removal of the jugular clamps. During the injection the blood pressure rose from 6 to 40 mm Hg.

Macroscopic examination. — No diffuse staining of the parenchyma was seen with the exception of C 312, which appeared stained faintly greyish blue throughout the brain. In C 102 and C 103, the vessels of the surface of the brains were stained. On the cut surface occasional blue streaks were seen in C 103 in the left cortex, the septum pellucidum and the thalamus bilaterally.

Microscopic examination. — In the pericallosal arteries the endothelial nuclei were stained in two brains. In the other brains the wall was as a rule unstained with an exceptional faint diffuse staining of the wall of the pericallosal arteries. The staining was most pronounced in C 103.

In 3 of the brains the intracerebral vessels were stained in the same way (see table 50), as a rule in the larger vessels. Sometimes a staining of the capillary wall was noted. In the capillaries no staining of the nuclei of the endothelial cells was seen. The parenchyma was not stained. The staining of the intracerebral vessels was most pronounced in the fore part of the brains in the cortex, the basal ganglia, the thalamus, the hypothalamus and the quadrigeminal bodies. In C 312 trpb remained in all the vessels, yet it was possible to see the staining of the vessel wall in larger vessels as a distinct and more intensely stained border.

Injection into common carotid artery with ligation of external carotid artery

Experimental. — In 3 animals the external carotid artery was ligated. In C 315 the occipital artery, which arose from the internal carotid artery, was ligated. Saline of 38° C and air, inserted in various amounts into the tubing of the cannula in the carotid artery, were injected into the left carotid artery with an application time of 40 seconds. The animal's head was turned to the left during the injection. The brain was cut, 3 sections being made from 4 parts of the brain.

Results

The data of the experiments are given in table 51. The amount of injected air was about 0.02, 0.2 and 1.0 ml, respectively. The application time was achieved. The blood returned to the pial vessels from 2 to 6 seconds after the end of the injection. The blood pressure before the injection varied from 55 to 90 mm Hg and fell about 20 mm Hg at most at the removal of the jugular clamps. The blood pressure increase during the injection varied from 20 to 44 mm Hg.

Macroscopic examination. — The areas normally permeable to trpb were stained. The dura mater was stained bilaterally. On the brain surface, C 315 was stained in the vessels and, diffusely stained occipitally, on the left side. C 265 was stained in the arteries of the right hemisphere and on the left side of the cerebellum. In C 267 the left hemisphere was stained blue with the exception of the area supplied by the pericallosal artery. The arteries of the right hemisphere and the cerebellum were stained blue.

On the sections C 265 was not stained but C 267 was stained diffusely blue, particularly in the cortex of the fore part on the left side and in the upper part of the corpora quadrigemina on both sides. In the cerebellum and dorsal part of the brain stem a dotted staining occurred.

Microscopic examination. — The pericallosal arteries were stained

in the nuclei of the endothelial cells. In C 265 some of the vessels of the parenchyma were stained in the same way. The walls of the capillaries were stained diffusely blue. This kind of staining of the parenchymal vessels occurred in the cortex on the right side of the fore part of the brain, in the dorsal part of the hippocampus and in the cerebellum. The parenchyma was not stained. In C 267 the staining of the vessels of the parenchyma was more disseminated, and diffuse parenchymal staining occurred in the cortex most pronouncedly on the left side, in the basal ganglia, septum pellucidum, cerebellum and brain stem. In the thalamus and the hypothalamus only staining of the vessels occurred. In C 315 larger branches of the parenchymal vessels were stained in the wall, as a rule also the endothelial nuclei. The capillaries as a rule showed a diffuse staining of the wall. The areas with blue staining on macroscopic examination showed definite diffuse staining in reflected light around the capillaries.

II. BODY TEMPERATURE 20°C

Injection into common carotid artery with ligation of external carotid artery

Experimental. — In order to investigate the risk of lesion by air embolism in hypothermia, 2 animals were cooled and air was injected after ligation of the external carotid artery. The air was injected into the tubing of the cannula in the left common carotid artery and the application time was 40 seconds. The animal's head was turned to the left during the injection. The intention was to kill the animals 30 minutes after the beginning of the trpb infusion. The brains were sectioned, 3 sections being made from 4 parts of the brain.

Results

The data of the experiments are given in tables 52 and 53. The animals were cooled to about 21.5° C in about 60 minutes. The rectal temperature at the injection was about 21° C. The injected amount of air varied from 0.2 to 1.0 ml. The decided application time of 40 seconds was achieved in both experiments. The saline was washed away by the blood 2 seconds after the end of the injection.

C 270 died when about three quarters of the trpb solution was infused but was not perfused with saline until 30 minutes after the beginning of the trpb infusion. The blood pressure was 40—55 mm Hg at the beginning of the experiment. In C 270 the blood pressure rose about 40 mm Hg during the injection.

Macroscopic examination. — The areas normally permeable to trpb

were stained. The dura mater was blue but was not stained on the left side in C 270. The vessels on the surface of the brain were stained. C 268 was faintly stained on the surface of both hemispheres. On the cut surface occasional faint blue streaks or dots were seen in the cortex, the hippocampus, the basal ganglia and the thalamus of both brains.

Microscopic examination. — The wall of the pericallosal arteries was stained. Occasionally the internal elastic membrane was stained. As a rule the nuclei of the endothelial cells were stained. In the parenchyma, the larger vessels were stained in the same way. The wall of the capillaries was often diffusely stained. The vessels were generally well rinsed. In the cerebellum and the occipital lobes trpb was seen in the lumen of the capillaries. No staining of the parenchyma occurred. The changes in the vessels were disseminated through the whole brain.

E. INFUSION OF TRYPAN BLUE AND RINSING WITH SALINE WITH AIR IN THE TUBING

Experimental. — Two rabbits were infused with trpb at normal body temperature. The rinsing was performed in the usual way except that the tubing to the aorta was not filled with saline below the air trap. The volume of air thus obtained in the tubing was calculated to be about 60 ml. In one of the animals an injection of saline of room temperature was made into the left common carotid artery. After removal the brain was cut, 3 sections being made from 4 parts of the brain.

Results

The experimental data are presented in table 54. The animals were killed 15 minutes after the beginning of the trpb infusion. After the rinsing with about 300 ml. of saline, which went in slowly as compared with such experiments in which air was evacuated from the tubing system the brains were fixed *in situ* with 200 ml of formalin. This infusion also went in more slowly than ordinarily.

Macroscopic examination. — The areas normally permeable to trpb were stained, including the dura mater. On the surface C 323 was faintly greyish blue bilaterally within the area of the middle cerebral artery and in the vermis cerebelli. C 330 was of the same appearance within the area of the left pericallosal artery and the cerebellum. On the cut surface C 323 appeared unstained but C 330 was greyish blue in the upper part of the quadrigeminal bodies, the cerebellum, and the medulla oblongata.

Microscopic examination. — The pericallosal arteries were not stained

in any of the brains. Within the macroscopic blue tinged areas the lumen of the vessels was as a rule still filled with trpb. In such areas occasional staining of the vessel wall was seen. In C 330 extravasal staining was seen on 2—3 occasions around larger vessels. The stained area was limited to the immediate neighbourhood of the vessel, which was filled with trpb. A staining of the vessel wall could not be excluded. No staining of the endothelial nuclei was observed.

DISCUSSION

The course of the experiments with intracarotid injection of saline at normal body temperature deserves a more detailed discussion on some points. It was evidently almost impossible to achieve the decided application time at injection into the common carotid artery of saline whether of room temperature or of body temperature. This was, however, regularly obtained in the cases with ligation of the external carotid artery and in hypothermia. It might be difficult to follow an injection mass of saline in the vessels under visible light, as pointed out by MARGOLIS, GRIFFIN, KENAN, TINDALL, RIGGINS & FORT (1959), in experiments on the circulation in the exposed spinal cord in the dog. These authors found that the head of the injection mass was sharply outlined when the saline was labelled with Na-fluorescein and studied in ultra-violet light. It seems, however, that such a difficulty might exist independently of the mode of injection. As an expelling of the blood by the saline is obtained initially and as the injection goes on at about the same or at an increased injection pressure, the return of blood in the pial vessels might be due either to an easier passage for some reason or other of the saline into the external carotid artery, or to a dilution of the injected solution by the blood that enters the circle of Willis from the contralateral internal carotid artery and the vertebral artery. The former alternative might be explained by the possibility that the vessels in the area of the external carotid artery dilates more than those of the internal carotid artery during the injection. However, the predetermined application time was obtained with saline on ligation of the external carotid artery at normal body temperature and at injection of saline into the common carotid artery in hypothermia. As the injection pressure was kept constant and the blood pressure was low in hypothermia the effective injection pressure is higher in these experiments. This might mean that the injected solution has a pressure head in the circle of Willis that is high enough to wash away the blood coming from the other tributaries of the circle of Willis. Also the possible vascular reactions may be less pronounced in hypothermia than at normal body tempera-

ture. On ligation of the external carotid artery, the pressure of the injected solution is higher at the anastomoses in the circle of Willis, than at injection into the common carotid artery as the injection pressure, which was the same, is distributed on a smaller vascular area. It is also possible that the fairly low viscosity of the saline is a factor reducing the possibilities of expelling the blood from the pial vessels during the whole injection, when the injection is made into the whole carotid artery system.

The possibility that an embolus in the internal carotid artery suddenly forms during the injection does not seem likely as the only possible source of such an embolus would be air in the injection system. Such air was carefully avoided. Furthermore, the experiments with injection of air do not lend any support to the suggestion that air is an obstacle to injection of saline.

The rise in the blood pressure during the injection, generally occurring during the latter part of the injection, was as a rule slight compared with the level before the injection. However, the rise occurs in spite of the loss of blood taking place at the same time. McDOWALL (1924) showed that a fall in venous pressure due to bleeding may lead to an initial rise of blood pressure. It has not been investigated in the present series of experiments whether the rise is due to the venous bleeding or to an effect of the saline on the carotid sinus or on the vasomotor centre. The blood pressure rise was not less pronounced at injection with ligation of the external carotid artery.

After the injection of saline the blood pressure fell, rose again during the infusion of trpb, and again fell towards the end of and after the infusion. The animals lived until 15 minutes after the beginning of the trpb infusion.

The amounts necessary for injection are smaller when the external carotid artery is ligated even if the application time is longer, as pointed out by STEINWALL (1958). This is obviously due to the smaller capacity of the internal carotid system as compared with the common carotid system.

The small amount might give a source of error when a substance is injected differing from the one which fills the injection system. The injected volume has to be larger than the capacity of the tubing to the common carotid artery. The variation in the injected amount that occurs on injection into the common carotid artery with ligation of the external carotid artery and with the same application time might be due to an anomalous origin of the occipital artery from the internal carotid artery.

The decided application time was achieved at injection into the common carotid artery at body temperatures of 25° C and 20° C with the exception of one experiment at 25° C.

In hypothermia the increase in the blood pressure during the injection was slight and about the same with both injection techniques, with the exception of one experiment when the injection was performed with ligation of the external carotid artery. In this experiment a considerable rise occurred. The blood pressure rise during the injection did not deviate perceptibly as compared with the experiments at normal body temperature.

The time for the return of the blood into the pial vessels after the end of the injection was considerably longer in hypothermia in the experiments with ligation of the external carotid artery. The application time was longer in the latter series of experiment and the blood pressure tended to be lower in that group. Both these factors might influence the time for the return of the blood.

The injected volumes were of the same magnitude in the series with injection into the common carotid artery and there was no difference between 20 and 25° C, nor between these series and the series at normal body temperature even if the variation was greater at this latter temperature.

The normally permeable areas (at 25° C only the choroid plexuses were registered) were blue in hypothermia independently of the length of the trpb duration and the circulatory status after the injection, except in the experiment with the very low blood pressure. In this experiment the choroid plexuses of the fourth ventricle were blue and the hypophysis and pineal body were faintly blue. The dura mater was ordinarily stained bilaterally only in one experiment, and was not stained in one animal. In the others the staining was generally normal on the right side and faint on the left side or somewhat less intense on the left than on the right side. Thus the normally permeable areas with the exception of the dura mater were stained in hypothermia when the circulatory status was not very poor. The more pronounced staining on the right side of the dura mater might be due to the ligation of the common carotid artery and a poor flow of blood from the other external carotid artery through the anastomoses owing to the low blood pressure.

The reason for the disadvantageous effect of the trpb infusion in hypothermia is not clear and will be more fully discussed further on.

Neither at normal body temperature nor in hypothermia was there any staining of the parenchyma in the experiments in which rinsing with saline was performed. Staining of the intracerebral vessels was only seen on very few occasions. Occasionally trpb was found in the lumen of capillaries, particularly in the cortex. It is a reasonable conclusion that the present technique did not give any injury to the BBB.

Microscopically the experiments without rinsing of the trpb showed

only staining of the vessel wall as rare exceptions. A few pinpoint large areas of extravascular staining were seen in hypothermia. Occasional staining of the pericallosal arteries was seen. Macroscopically the brains had an appearance that could not always be distinguished from a slight injury to the BBB, as pointed out by BROMAN (1950).

BJERNER, BROMAN & SWENSSON (1944), who performed *post mortem* rinsing of the vessels, found no passage of trpb into the brain of normal rabbits. However, in normal rats occasional dotted staining of the brain parenchyma could be found. CLEMEDSON, HARTELIUS & HOLMBERG (1958) found such lesions in 3 out of 18 normal rabbits that were infused with trpb and then rinsed *post mortem* at normal body temperature.

Staining of the pericallosal arteries had a tendency to occur faintly and irregularly when no *post mortem* rinsing was performed.

WILLIAMS (1949) showed in the mouse and rat that the internal elastic membrane of the extracranial arteries was stained by trpb given in repeated doses during a period of days. The central nervous system was not thoroughly studied but faint staining of the internal elastic membrane of the small cerebral arteries was reported. KING (1938 b) found a staining of the internal elastic membrane of the cerebral arteries of the mouse after intravenous injection of trpb. He found, however, that the staining was not pronounced until 24 hours had elapsed in contrast to the same kind of staining in the extracranial arteries, which was seen 10 minutes after the injection of trpb. BROMAN (1949) reported that the circle of Willis was stained a slight but distinct blue in normal animals. The staining faded away in the branches of the circle of Willis. The basilar artery was not stained. It seems as the extra cerebral vessels can be stained to a varying degree and extent in different animals both in acute and chronic experiments with trpb.

The possibility that remnants of trpb in the vessels can give artefactual staining, has been investigated by BROMAN (1950). He found that the brain parenchyma of animals (rabbits and cats) perfused with trpb *post mortem* and rinsed with formalin was stained, which was checked on microscopic investigation. In another series of experiment the animals were infused with trpb *in vivo* and rinsed with saline at increasing intervals after death. BROMAN found that after 2 hours the brains were macroscopically stained in spite of the rinsing, but on microscopic investigation were shown to contain dye in the vessels and not in the parenchyma. He concluded that on defective rinsing of the vessels macroscopic investigation alone could be misleading, a point of view confirmed by BROMAN, RADNER & SVANBERG (1950) on human brains stained supravitaly. In experiments with cerebral emboli on dogs, SWANK & HAIN (1952) used a saturated trpb solution without further reports on the con-

centration. In the non-injected side of the cerebrum no passage of trpb into the parenchyma was noted. The brains were not fixed *in situ*. CULLEN & SWANK (1954) infused a 13 per cent trpb solution into hamsters without rinsing of the vessels and found no passage of trpb into the brain parenchyma in control animals. In experiments with bromphenol blue in guinea pigs BROMAN (1938) gives evidence that this dye, when not rinsed from the vessels *post mortem*, passes into the brain parenchyma and concludes that the vessels should be rinsed as soon as possible after death since otherwise undamaged parts of the brain may be stained.

The results of the present experiments with infusion of trpb *in vivo* in rabbits without rinsing of the vessels are in agreement with those of BROMAN (1950). The few areas with passage of trpb into the brain parenchyma in hypothermia in 2 of the experiments do not invalidate the conclusion that trpb left in the lumen of the vessels generally does not pass into the brain parenchyma in visible amounts. It might be added that CLEMEDSON, HARTELIUS & HOLMBERG (1958) found passage of trpb into the brain parenchyma of normal rabbits after rinsing. The possibility of a variation in the animal with regard to the BBB cannot be excluded. Such a variation might be due to constitutional or acquired factors. The most common lesion in the brain of a normal rabbit is a spontaneous granulomatous encephalomeningitis (LORENTZEN, 1950; BERG & LINDGREN, 1958). In an investigation of the BBB with radioactive bovine albumin as an indicator of the lesion, LEE & OLSZEWSKI (1959a) found granulomas in several rabbits but no radioactivity corresponding to these lesions. Three brains which showed a few areas of parenchymal staining in the experiments with no rinsing of the trpb and were investigated with regard to the occurrence of spontaneous encephalomeningitis showed no such lesion.

The main effect of rinsing with formalin instead of saline was that trpb was not rinsed from the vessels. No substantial difference was found between the experiments at normal body temperature and in hypothermia (20° C) in this respect. The results of BROMAN (1950) in supravital staining were thus not found to be valid when the trpb was infused *in vivo* in experiments on adult animals.

In an investigation of the BBB of fetuses of rabbits and newborn guinea pigs, GRÖNTORT (1954) established the following criteria of artefactual staining of the brain in animals supravitaly stained and then rinsed and fixed with formalin with the brain *in situ*: If the vessels contain trpb in the lumen and the parenchyma is diffusely stained without staining of the parenchymal nuclei, the lesion is to be regarded as artefactual. BROMAN's (1950) experiments on supravital staining of adult animals seem to support such a view.

The experimental findings presented here do not imply that the criteria of GRÖNTOFT (1954) of artefactual staining are valid with the technique used in the present investigation on living animals.

In the series of experiment with injection of air in saline it was found that only staining of the vessel wall could occur. On injection with ligation of the external carotid artery, however, a staining of the parenchyma also occurred. The results seem to indicate that a smaller amount of air is necessary for provoking an impairment of the BBB when the external carotid artery is ligated.

The few experiments that have been carried out do not give any conclusive information with regard to the effect of hypothermia on injury to the BBB produced by intracarotid injection of air. They inform us, however, that the possibility of artefactual staining from air embolism in hypothermia of 20° C does exist. The information given by the experiments as regards air in the injection system as a source of error is that relative large amounts of air are necessary for provoking lesions on injection into the common carotid artery and that the damage might be confined to the vessel wall. POLLARD, FLEISCHAKER, TIMMES & KARLSSON (1961) found on extracorporeal cooling in the dog that sodium fluorescein could pass out into the brain parenchyma, which was presumably due to air embolism.

An air bubble so large that it may be seen on inspection of the injection system before the injection did not give any abnormal staining in the brain on injection into the common carotid artery. Such air bubbles were always removed in the present experiments.

The previous investigation of the effect of air on the BBB by BROMAN (1938, 1940a), ROZDILSKY & OLSZEWSKI (1957) and LEE & OLSZEWSKI (1959b) on injection into guinea pig, cat and rabbit, indicate that smaller amounts of air were necessary to provoke an injury to the BBB than in the present investigation under comparable conditions, i.e. injection into the common carotid artery. This might be due to the different technique, such as injection of the air as foam (BROMAN 1938, 1940a) or another indicator of the injury to the BBB than trpb (ROZDILSKY & OLSZEWSKI, 1957; LEE & OLSZEWSKI, 1959b) or to species difference. The lesion produced in rabbits by LEE & OLSZEWSKI (1959b) with trpb as indicator of the injury to BBB was only slight. The injection was made into the common carotid artery. The present experiments with selective injection into the internal carotid artery showed that also small air bubbles in the tubing in combination with air bubbles in the syringe might provoke a pronounced lesion of the BBB.

According to BROMAN (1938, 1940a, 1949) the macroscopic appearance of the lesion produced by air foam could be punctate, patchy and occa-

sionally uniform. LEE & OLSZEWSKI (1959b) observed that the brain was slightly blue tinged. In the experiments with radioactive iodinated bovine albumin (ROZDILSKY & OLSZEWSKI, 1957; LEE & OLSZEWSKI, 1959b) found the lesion to be patchy. In the present experiments the macroscopic appearance was one of pronounced staining of the vessels. In cases in which the parenchyma was stained this was confined to one part of the brain. The staining consisted of fairly large patches with a tendency to confluence.

The microscopic appearance has been described by BROMAN (1940a) only and consisted of staining of the vessel wall. Trpb was as a rule seen around these vessels. In the present investigation the staining of the vessel wall could be very pronounced with staining of the endothelial nuclei without any passage of trpb into the brain parenchyma. When such passage occurred the staining was diffuse with almost no staining of the parenchymal nuclei.

The experiments with rinsing with saline, the tubing being below the air-trap filled with air (about 60 ml), showed no such lesion as seen on injection into the carotid artery. The main effect of the air was defective rinsing.

SUMMARY

The control of the technique with injection of saline instead of Umbradil under the various experimental conditions showed that no injury to the BBB was obtained.

Evaluation of certain possible sources of error in the method supposed to be capable of giving artefactual staining revealed that both at normal body temperature and in hypothermia, trpb remnants in the vessel or rinsing with formalin might only rarely give a few pin-point areas of extravascular staining.

The risk that air embolism on intracarotid injection gives artefactual staining seemed to be somewhat increased when the external carotid artery was ligated. The risk of air embolism also existed in hypothermia.

The lesion produced by air embolism was characterized by an intense staining of the vessel wall, particularly the endothelial cell nuclei, as a rule without staining of the parenchyma.

At normal body temperature, air in the rinsing system seemed to give only defective rinsing even in large amounts.

AREAS IN THE BRAIN NORMALLY PERMEABLE TO TRYPAN BLUE

Brief descriptions of the areas of the brain normally permeable to trpb have been given by SPATZ (1934), KING (1938a) and BAKAY (1956).

GOLDMANN (1909, 1913) noted that although the brain parenchyma was not stained by trpb, repeatedly injected for a length of time, either subcutaneously or into the blood stream, the choroid plexuses and the dura mater were stained blue. This observation has since been confirmed by a number of workers, e.g. RACHMANOW (1913), BEHNSEN (1927), MANDELSTAMM & KRYLOW (1928), SPATZ (1934) and WISLOCKI & LEDUC (1952).

A faint and often irregular staining of the pia mater was reported by GOLDMANN (1913), RACHMANOW (1913) and MANDELSTAMM & KRYLOW (1928).

The vital staining of the hypophysis was shown by SCHULEMANN (1912) and confirmed by GOLDMANN (1913), RACHMANOW (1913), BEHNSEN (1927), MANDELSTAMM & KRYLOW (1928), WISLOCKI & KING (1936) and WISLOCKI & LEDUC (1952).

RACHMANOW (1913) described the vital staining of the tuber cinereum later confirmed by BEHNSEN (1927), MANDELSTAMM & KRYLOW (1928) and SPATZ (1934). The part of the tuber cinereum which was stained with vital dyes was more closely defined by WISLOCKI & KING (1936) as the nearest surroundings of the hypophysial stalk called by them the eminentia sacularis, in modern terms the median eminence (RIOCH, WISLOCKI & O'LEARY 1940).

The staining of the pineal body was demonstrated by BIONDI (1916) and later on reported by MACKLIN & MACKLIN (1920), MANDELSTAMM & KRYLOW (1928) and WISLOCKI & LEDUC (1952).

The area postrema was described to be stained with trpb by WISLOCKI & PUTNAM (1920), BEHNSEN (1927), MANDELSTAMM & KRYLOW (1928) and WISLOCKI & LEDUC (1952).

PUTNAM (1922) described a structure in the anterior wall of the third ventricle of the mammalian brain located between the columns of the fornix on a level with the upper border of the foramina of Monroe, which he called the intercolumnar tubercle. The structure has also been referred to as the paraphysis (BEHNSEN, 1927) and the subfornical body

(CAMMERMEYER 1947). The intercolumnar tubercle is stained by trpb (PUTNAM, 1922; BEHNSEN 1927; MANDELSTAMM & KRYLOW, 1928).

An area in the anterior wall of the third ventricle above the chiasma, the recessus opticus or supraoptic crest, was found to be stained by trpb (BEHNSEN 1927; MANDELSTAMM & KRYLOW, 1928; WISLOCKI & KING, 1936; WISLOCKI & LEDUC, 1952).

The results mentioned above were obtained by repeated injections of trpb for a period of time, the exception being SPATZ (1934) who injected the trpb solution in a single intravenous dose and killed the animals 6 hours later.

The staining of the brain, however, has also been studied in short-term experiments, in which the animals have got large doses of trpb intravenously and been killed within 1—2 hours.

WISLOCKI & KING (1936) studied the staining of the median eminence after intravenous injection of 20 ml of a 2 per cent trpb solution. The staining was most pronounced after 45 minutes and had almost faded away after 24 hours.

In experiments with intravenous injection of trpb in mice and guinea pigs, examined from 10 minutes to 72 hours afterwards, KING (1938b) found staining of the dura mater, the choroid plexuses and the pineal body.

BROMAN (1940c) reported that in short-term experiments the vessel wall and the epithelial cells of the choroid plexuses were stained a diffuse blue by trpb.

The staining of the areas normally permeable to trpb in short-term experiments was confirmed by GRÖNTOFT (1954) in newborn guinea pigs and in the foetus of rabbits. In experiments with supravital perfusion of the trpb in human foetus he reported the staining of the choroid plexuses, the infundibulum and the hypophysis.

In long-term experiments the trpb is as a rule found as granules within the cells, in short-term experiments the staining is generally diffuse. On reviewing the literature it was found that in the long-term experiments a detailed account of the microscopic picture was given, which was almost lacking in the description of the results obtained by staining in short-term experiments.

The permeability of the above-mentioned areas, in contrast to the brain proper, to other substances than trpb has also been reported, e.g. Br⁸² (GRUNER, SUNG, TUBIANA & SEGARRA, 1951), silver (WISLOCKI & LEDUC, 1952; DEMPSEY & WISLOCKI, 1955), proteins in the choroid plexuses, area postrema, intercolumnar tubercle and supraoptic crest (LEDUC, 1955), P³² in the hypophysis, infundibulum, pineal body, area postrema and choroid plexuses (see BAKAY, 1956), radioactive iodinated

bovine albumin in the hypothalamus and choroid plexuses (LEE & OLSZEWSKI, 1959a), N-acetyl-4-aminoantipyrine and sulfaguanidine in the hypophysis, intercolumnar tubercle and area postrema (WILSON & BRODIE, 1961).

In hypothermia the staining of the areas normally permeable to trpb has been investigated by LOURIE, WEINSTEIN & O'LEARY (1960). They used the presence of blue-staining of "the hypophysis, epiphysis, choroid plexus, area postrema, etc (WISLOCKI & LEDUC, 1952)" as a criterion that the dose of trpb had been adequate in their study of the effect of hypothermia on vital staining of the brain. However, no detailed account of the results of the staining of these areas was given and it was not clear to what extent, if any, hypothermia might influence the staining of these areas.

The present investigation was performed in order to obtain information on the staining of the areas normally permeable to trpb in acute experiments in hypothermia. As control a similar investigation of normothermic animals was carried out. This material was also used for a study of the microscopic picture of the staining of the relevant areas in short-term experiments, on which only scanty information is available in the literature.

Methods

Sixteen rabbits were used, equally distributed over experiments at normal body temperature and in hypothermia (20° C). The animals were trephined on the left side of the skull, tracheotomized and artificially respired. A femoral vein and artery were cannulated for infusion of trpb (100 ml of a 0.5 per cent solution) and blood pressure recording, respectively. At each body temperature the animals were divided into 3 groups with a trpb duration of about 15, 30 and 60 minutes, respectively. *Post mortem* perfusion of the vessels with saline was carried out, followed by fixation *in situ* with formalin.

On removing the brain of rabbits, the median eminence is very frequently torn away and remains attached to the posterior lobe of the hypophysis (HARRIS, 1947). Therefore the frontal part of the brain was carefully lifted at removal of the brain and the hypophysial stalk was cut with a pair of small scissors. With this procedure it was generally possible to remove the median eminence together with the brain.

Four parts of the brains were cut, care being taken to secure the supraoptic crest, median eminence, pineal body and choroid plexuses. The area postrema and the hypophysis were sectioned separately.

No attempt was made to investigate the intercolumnar tubercle since this structure, examined in 4 brains (see chapter III), was found to be stained in one animal only.

Results

Body temperature 38° C.

The data of the experiments are given in table 55. The course was uneventful. The blood pressure, which was about 100 mm Hg, rose during the first part of the trpb infusion and generally fell during the later part of the infusion. In one animal the blood pressure was about 50 mm Hg. After the end of the infusion the blood pressure as a rule rose again.

Macroscopic examination. — The supraoptic crest, median eminence, choroid plexuses, pineal body, hypophysis and the area postrema were blue. The dura mater was stained blue bilaterally. No obvious difference was seen in intensity of staining with the variation of the trpb duration. The arachnoid over the basal cisterns was often slightly blue-tinged. The brain was not stained in any experiment.

Microscopic examination. — The parenchyma of the brain was not stained. The wall of the pericallosal arteries was not stained. The lumen of the intracerebral vessels was occasionally seen to contain trpb, particularly in the cortex. No positive proof of staining of the wall was obtained.

The choroid plexuses were stained in the vessel wall. The epithelium was diffusely stained. Only occasionally could staining of the nuclei of the epithelial cells be seen. The vessel wall of the supraoptic crest was stained and as a rule a faint diffuse staining around the vessels was seen. The median eminence was diffusely stained over an area occasionally extending into the lateral walls of the third ventricle. There was occasional staining of the vessel wall. Staining of the parenchymal nuclei of the median eminence was a rare exception.

The hypophysis was diffusely stained both in the neural and the glandular part. Staining of the parenchymal nuclei was exceptional. The pineal body was stained more intensely in the external part consisting of a brim surrounding the internal part of the pineal body. Occasional diffuse staining of the walls of the larger vessels was seen. The area postrema was as a rule the most faintly stained of the actual areas as the staining, which was diffuse, was seen regularly only in reflected, but not transmitted, light. Staining of the vessel wall was rarely seen. The staining was confined to the roof of the central canal.

Body temperature 20° C.

The cooling data are given in table 56. The cooling in water was stopped after about 80 minutes and the infusion started at about 110 minutes after the beginning of the cooling.

The mean rectal temperature was 20.5°C at the beginning of the trpb infusion. It rose about 0.5°C towards the end of the experiment.

The blood pressure was about 20 mm Hg before the infusion and mostly rose during the infusion (table 57). Towards the end of the experiment the blood pressure fell again. Three of the animals died during or just after the completion of the trpb infusion, about 10—15 minutes after the beginning of the infusion. The other five lived 30—60 minutes after the beginning of the trpb infusion, which as a rule lasted about 10—15 minutes. In one experiment the infusion of trpb lasted for 45 minutes.

Macroscopic examination. — The supraoptic crest, median eminence, choroid plexuses, pineal body, hypophysis and the area postrema were blue. The dura mater was faintly stained in C 245 and C 250, which died during the trpb infusion or immediately afterwards. In the other animals the dura mater was blue bilaterally. The brains were not stained, but in C 247 the vessels on the surface of the brain were blue and on the sectioned surface the basal ganglia, cortex, cerebellum and medulla oblongata appeared faintly stained.

Microscopic examination. — The brains were not stained in the parenchyma. Exceptionally the vessel wall could be stained in rinsed capillaries and in vessels larger than capillaries. On microscopic examination both in reflected and transmitted light the staining of that part of the brain of C 247 which was macroscopically faintly blue, proved to be due to trpb remaining in the capillaries.

The vessel wall and the cubic epithelium of the choroid plexuses were diffusely stained. Occasionally some nuclei of these cells were stained. Often round cells close to the vessel wall were stained. No staining of the endothelial nuclei occurred.

The outer brim of the epiphysis was diffusely stained. The vessel walls were as a rule not stained. The area postrema was mostly faintly stained. Occasionally the walls of the vessels were stained. In one case the parenchymal nuclei were stained. The staining was confined to the roof of the central canal. The hypophysis was stained both in the anterior and posterior lobe. The staining was more pronounced in the anterior lobe, in which there was occasional staining of parenchymal nuclei.

The median eminence was stained diffusely blue and the wall of the vessels sometimes appeared stained. There was occasional staining of the parenchymal nuclei. The staining was confined to the floor of the third ventricle but could be seen in the lateral walls. The vessel wall of the supraoptic crest was stained and occasionally the surrounding parenchyma was diffusely stained.

No staining of the nuclei of the endothelial cells was seen in those areas in which staining of the vessel wall occurred.

The intensity of the staining did not seem to increase with prolonged action of trpb.

Discussion

The areas of the brain normally permeable to trpb which were examined did not show any difference in type or degree of staining at the 2 different body temperatures, 38° C and 20° C. The length of the action of trpb before death did not influence the degree of staining to an extent noticeable with the method used.

There were only minor differences between the extent of staining if the results of the present experiments are compared with the reported staining on injection with trpb in small repeated doses for longish periods of time.

No comparison of the extent of staining in particular cells has been attempted, since the histologic technique used (unstained sections) does not admit of any detailed differentiation of the various cells, and must be limited to whether the vessel wall or the parenchyma was stained.

The choroid plexuses were diffusely stained in the vessel wall and in the epithelium as described by BROMAN (1940c) in similar experiments. In vital staining with trpb in long-term experiments the staining of the vessel wall did not seem to be a prominent feature in contrast to the staining of the epithelium (GOLDMANN, 1909, 1913; RACHMANOW, 1913; MANDELSTAMM & KRYLOW, 1928; WISLOCKI & LEDUC 1952).

The staining of the supraoptic crest was mainly confined to the wall of larger vessels. In long-term experiments with trpb in the rabbit, however, WISLOCKI & KING (1936) found the dye in the parenchymal cells and in macrophages surrounding the capillaries. In the rat WISLOCKI & LEDUC (1952) found trpb in the parenchyma in that part of the supraoptic crest which faces the third ventricle.

Thus the extent of the staining of the supraoptic crest is more limited in short-term than in long-term experiments. In an exhaustive description of the vascular anatomy of the supraoptic crest (*organon vasculosum laminae terminalis*) in the rabbit, MERGNER (1959) and WISLOCKI & KING (1936) distinguished a primary vascular plexus located beneath the pia mater and a secondary vascular plexus located beneath the ependyma of the third ventricle. The vessels that were stained in the present experiments seem to belong to the primary plexus as they were located just above the chiasma and were visible from the outer surface of the brain. In that part of the lamina terminalis which faces the ventricular lumen no blue-staining was seen, which is at variance with the findings of WISLOCKI & KING (1936) in long-term experiments on the rabbit.

RIOCH, WISLOCKI & O'LEARY (1940) distinguished the median eminence

from the tuber cinereum by the microscopic picture with tufted capillaries and cells of a particular type (Gersh's cells) and the characteristic vital staining. SPATZ, DIEPEN & GAUPP (1948) found that the part of the tuber cinereum which has special permeability properties was macroscopically separated, by a furrow, in the rabbit, from the remainder of the tuber cinereum. They also stated that this area was microscopically characterized by a loose glial syncytium with an intervening space filled with an amorphous structure and special glial cells (pituicytes). In the present experiments the staining occasionally extended across the furrow. The parenchyma was mainly diffusely stained. In long-term experiments the staining of the parenchymal cells was a prominent feature (RACHMANOW, 1913; MANDELSTAMM & KRYLOW, 1928; WISLOCKI & KING, 1936).

The hypophysis was mainly stained diffusely in both the neural and the glandular lobe. In essence similar results were obtained in long-term experiments with trpb (SCHULEMANN, 1912; WISLOCKI & KING, 1936; WISLOCKI & LEDUC, 1952).

The pineal body was stained diffusely and in a more pronounced way in the periphery of the organ. In long-term experiments BIONDI (1916) found the trpb located in the cells of the epiphysis of the rabbit with no predilection for the periphery. WISLOCKI & LEDUC (1952) found trpb in macrophages along the blood vessels and occasionally in the cytoplasm of the endothelial cells. In an electron microscopic investigation DEMPSEY & WISLOCKI (1955) found the silver in the endothelial basement membrane and occasionally in the pineal cells.

The area postrema was rather faintly stained as compared with the other areas of the brain permeable to trpb. The staining of the vessel wall did not occur regularly. In long-term experiments with trpb, however, WISLOCKI & PUTNAM (1920) and WISLOCKI & LEDUC (1952) found the dye in the connective tissue cells which invest the small arterioles and the capillaries. The endothelium of the vessels was rarely stained. In similar experiments WISLOCKI & PUTNAM (1924) found Prussian blue in the tissue spaces between the neuroglial cells. The macroscopic location of the staining in the present experiments is at variance with the findings of WISLOCKI & PUTNAM (1920), who found a stained area bilaterally in the caudal part of the fourth ventricle in one rabbit in long-term experiments with trpb. However, MOREST (1960) states that the area postrema in rodents is an unpaired midline structure overlying the central canal at the obex and thus differs from cat and man (KING 1937; CAMMERMEYER, 1944, 1947), where the area postrema is paired and, converging in the midline, forms the roof of the central canal. It seems more reasonable to assume that the difference referred to is due to a possible

variation of the anatomy of the area postrema rather than to a difference in the mode of action of trpb in short-term and long-term experiments. In a study of the accumulation of silver in long-term experiments in rat and hamster, WISLOCKI & LEDUC (1952) showed a figure with staining of the roof of the central canal. Neither in that paper nor in a similar investigation by DEMPSEY & WISLOCKI (1955) is special attention paid to the extent of the staining of the area postrema.

The intercolumnar tubercle which was found stained in one out of 4 brains examined in this respect, has also been reported to show irregular staining in long-term experiments with trpb in the rabbit (MANDELSTAMM & KRYLOW, 1928). PUTNAM (1920) examined rat, guinea pig, rabbit, dog and monkey and found an individual variation in depth of staining. In the rat and hamster, however, WISLOCKI & LEDUC (1952) did not seem to have observed any irregularity of the staining of the intercolumnar tubercle.

The dura mater, which was not examined microscopically, showed blue-staining in the short-term experiments in agreement with the results obtained by repeated injection of trpb in small doses during a longish period (GOLDMANN, 1909, 1913). In hypothermia, however, the staining of the dura mater was not as pronounced as it was at normal body temperature when the action of trpb was short.

LOURIE, WEINSTEIN & O'LEARY (1960) seemed to obtain staining of the normally permeable areas in hypothermia with such a small dose as 4 ml of one per cent trpb solution given intravenously and with effectively maintained circulation for at least 10 minutes in animals with a brain temperature of 9° C and a rectal temperature of about 25° C. Also on supravital perfusion (40 ml of a 0.15 per cent trpb solution) of cats cooled to a brain temperature of 2.5 to 8° C, the areas normally permeable to trpb seemed to be stained. It does not seem likely that the dura mater has been included in these areas in view of the results of the staining of the dura mater obtained in the present investigation.

WISLOCKI & LEDUC (1952) established that the major difference between the storage of silver and trpb in the relevant areas of the brain was that the former mainly accumulated extracellularly, whereas trpb was as a rule deposited intracellularly. In an investigation by electron microscope using silver as the vital stain, DEMPSEY & WISLOCKI (1955) reported that in the choroid plexuses, area postrema, intercolumnar tubercle, neurohypophysis and the pineal body, silver was deposited in the form of dense granules in the basement membrane upon which the capillary endothelium rests. Furthermore the silver was found in a loose pericapillary sheath and in an outer membrane separating this sheath from the parenchymal cells.

The factors are unknown which make it possible for trpb to permeate into the above-mentioned areas in contrast to the brain proper. Several hypotheses have been suggested. It seems clear that these areas differ in their structure from the rest of the brain with regard to the presence of sinusoidal blood vessels surrounded by conspicuous connective tissue sheaths (see WISLOCKI & LEDUC, 1952). KING (1938a, b) attributed the staining of these areas to the presence of connective tissue for which he stated that trpb had a high affinity. In their electron microscopic investigation DEMPSEY & WISLOCKI (1955) found that, in contrast to the rest of the brain, the areas with normally increased permeability were characterized by a doubling of the capillary basement membrane, so that the glial end-feet were attached to the outer membrane at a distance from the capillary wall instead of to the inner membrane as in the rest of the brain. The space between the membranes consisted of connective tissue cells and fibres, in which the silver was deposited. In an electron microscopic investigation of the brain in the rabbit, mouse and cat LUSE (1960b) found that the glial processes completely surrounded the vessels, whereas in the hypothalamus of a mouse and in the neurohypophysis of a cat there was an interstitial space around the capillary.

Whatever factors facilitate the transport of trpb into the above-mentioned areas or prevent it from entering the rest of the brain, it is shown by the present investigation that the staining of these areas with the exception of the dura mater is not perceptibly interfered with by the changes in normal physiology caused by hypothermia.

Summary

The staining of the areas normally permeable to trpb was not perceptibly interfered with in hypothermia. The dura mater might be more faintly stained in hypothermia than in normothermia when the action of trpb was of short duration. Only minor differences existed between the extent of staining of the actual areas in short-term and long-term experiments.

INJURY TO BLOOD-BRAIN BARRIER PRODUCED BY UMBRADIL IN NORMOTHERMIA

Studies on the effect of roentgen contrast media on the BBB began with the work of BROMAN & OLSSON (1948). In this and subsequent papers (BROMAN & OLSSON, 1949, 1956a; BROMAN, FORSSMAN & OLSSON, 1950) they reported the method used and the results under varying experimental conditions. Their method has been described in chapter I.

BROMAN & OLSSON (1948, 1949) tested in rabbits various iodopyracet compounds such as Umbradil, Umbradil forte, Umbradil-methylglucamine, Dijodon and Diodrast. They found that Umbradil 35 % did not give any lesion with application times of 30 to 40 seconds. Dijodon 35 % and Diodrast 35 %, chemically identical with Umbradil 35 %, however, produced lesions with application times of 10 to 20 seconds. Umbradil 50 % damaged the BBB with an application time of 10 seconds. The lesion was regularly observed with application times of 15 to 40 seconds. Umbradil forte 50 % gave a slight lesion with an application time of 15 seconds. Dijodon 50 % and Umbradil-methylglucamine 50 % produced pronounced lesions with an application time of 10 seconds. Umbradil 70 % damaged the BBB in application times of 1 to 2 seconds.

Convulsions during the injection of the contrast medium were often noted.

The lesion was as a rule located in the hemisphere on the side of the injection. Exceptionally it occurred, however, that the convexity of the hemisphere on the side of injection was not damaged though the contralateral hemisphere showed a pronounced staining. In such experiments the centre of the hemisphere on the injected side was as a rule distinctly stained.

By infusing Congo red immediately after the injection of the contrast medium and trpb after a varying length of time it was found that the damage to the BBB was seen distinctly one hour after the injection but not after two hours. The damage was thus acute and its extension was assumed to represent a vascular region in which an injurious concentration had been attained. The authors' opinion was that the damage represented a pure injury to the BBB without edema, hemorrhages, necrosis of the tissues or thrombosis. However, in severe lesions edema was noted and also punctate hemorrhages and stasis. The severity of the lesion

varied according to the application time and the concentration of the contrast medium.

In order to elucidate the pathogenesis of the lesion produced by the contrast media, glucose and sodium chloride were injected in such concentrations that the same, or higher, osmotic pressure as that of the contrast media was achieved. In these experiments, lesions were obtained but were not as pronounced as those produced by the contrast media.

BROMAN, FORSSMAN & OLSSON (1950) investigated the effect of repeated injections of contrast media in non-injurious doses or in combination with another injurious agent in non-toxic doses.

It was found that repeated injections of Umbradil 35 % gave lesions with an interval of 1 to 3 minutes but not with an interval of 15 minutes. A preceding injection of 1.6 ml 10 per cent formalin or of sodium taurocholate solved to 1 per cent in Umbradil 35 % made the injected solution injurious.

In order to study the effect of the length of the application time when the injected amount of contrast medium was kept constant, the cerebral arteries were clamped during the injection but the venous outflow from the brain was kept intact. It was found that a contrast medium, which did not upset the BBB with an application time of 10 seconds, became injurious when the application time was prolonged to 25 and 37 seconds.

BROMAN & OLSSON (1956b) found that Triurol 25 % damaged the BBB to the same degree as Umbradil 35 %.

BLOOR, WRENN & HAYES (1951) studied the effect of Diodrast 35 % and 70 % after injection by means of a catheter placed in the common carotid artery at the ostium of the internal carotid artery in rabbits. The application time was not recorded. The damage to the BBB was shown with the tetra-sulfonic-acid of copper phtalo-cyanine. The animals were curarized and artificially respiration. The electrocorticogram was recorded. 0.8—4.8 ml of the contrast medium was injected at a rate of 0.4 ml/2—3 sec. The indicator solution of the disturbance of the BBB was injected into the internal carotid artery or intravenously. After the solution had been given 45 minutes to take effect, the animal was killed. It was not mentioned whether *post mortem* perfusion with saline was performed. All doses of the contrast medium provoked changes in the EEG. But only 4.8 ml of Diodrast 35 % and 0.8 ml of Diodrast 70 % gave disturbance of the BBB. The EEG changes consisted of depression of all cortical activity and general or focal delta activity. Disturbance of the BBB occurred almost exclusively in the group with depressed cortical activity.

BLOOR, WRENN & MARGOLIS (1951) performed further experimental investigations with the technique described above. The contrast media

were injected via the catheter over 30 seconds when non-divided doses were used. Diodrast, Urokon, its non-iodinated homologue, N-methyl nicotinamid HCl and paraamino-hippurate were particularly studied. In accordance with BROMAN & OLSSON (1948, 1949) and BROMAN, FORSSMAN & OLSSON (1950) it was found that the concentration is a crucial factor in the dosage of contrast media. The lesion decreased in frequency if carbogen (10 % CO₂ and 90 % O₂) was given. The non-iodinated compound of Urokon gave a disturbance of the BBB.

The histopathologic studies in these short-term experiments showed that the changes indicated by the macroscopic staining and the EEG abnormalities ran parallel with the microscopic changes. The vascular injury, edema and staining were almost entirely confined to the gray matter, cortex and deep nuclear masses. Vasodilatation and staining appeared as early as one minute after the injury. The early changes consisted of a uniform dilatation of parenchymal vessels of all calibers. At a later stage stasis became evident. Ball-shaped hemorrhages of varying sizes were almost always present in the specimens representing significant alteration of the vessels. Edema was seen as a characteristic swelling of the oligodendroglia. Evidence of staining of the intracerebral vessels with the dye was never present in the normal brain. The tissue affinity for the dye appeared in the following descending order: Endothelial cell nuclei, neurones and glia. The authors explained the partial protection afforded by inspired CO₂ by the "diluting and washing out effect" following increased cerebral blood flow. The authors concluded that a larger amount of contrast medium is tolerated when administered in small doses — the longer the period of time between injections, the greater the tolerance. This is in accordance with the findings of BROMAN, FORSSMAN & OLSSON (1950).

SMITH *et al.* (1951) produced disturbance of the BBB by injecting Diodrast 70 % into the common carotid artery of rabbits. The injection pressure was recorded and kept low. A measured amount of Diodrast was injected and the injection time recorded. Three minutes after the end of the injection, trpb or disulphine blue or sodium fluorescein was infused intravenously. The animals were killed after a varying length of time. Nothing is mentioned about application time or *post mortem* rinsing of the vessels. The injection time was fairly long, the shortest 8 ml in 45 seconds and the longest 10 ml in 10 minutes. The pial vessels were dilated as they were filled with Diodrast. Some of the animals lived 1, 3 or 6 hours before the indicator dye was injected. The damage did not persist over 3 hours.

BASSETT, ROGERS, CHERRY & GRUZHIT (1953) investigated the effect of Diodrast, Urokon, thorium dioxide, sodium iodide, sodium chloride

and glucose solution with the same as or greater osmotic pressure than Diodrast 35 % on the BBB, especially in dogs. The injury to the BBB was indicated by the passage of Evans blue into the brain parenchyma. The injections were made into the common carotid artery. In some experiments the Evans blue was injected before the contrast medium.

The authors concluded that their results were in agreement with those of BROMAN & OLSSON (1948, 1949). They found, however, that as a rule the lesion occurred already with an application time of 10 seconds for Diodrast 35 %. They also observed a blood pressure fall and a rise in the cerebrospinal fluid pressure during the intracarotid injection of Diodrast. Glucose and saline solutions of the same osmotic pressure as Diodrast 35 % also gave lesions but not of the same degree as those obtained with the contrast medium. No histologic changes were seen in the brain of animals that were allowed to live for a certain time after injection of Diodrast 35 % in an amount causing damage to the BBB.

HOL & SKJERVEN (1954) showed that with the rabbit in a supine position the spinal cord was stained by trpb after injection of Nycotrast 70 %.

MARGOLIS, TINDALL, PHILLIPS, KENAN & GRIMSON (1958) found histopathologic changes in the spinal cord on injection of roentgen contrast media into the abdominal aorta in dogs in the supine position. The histopathologic changes varied from scattered microscopic foci of destruction affecting gray or white matter to a complete transverse necrosis of the spinal cord. The transition from moderate to severe lesions was abrupt.

TINDALL, KENAN, PHILLIPS, MARGOLIS & GRIMSON (1958) investigated the effect on the spinal cord of Diodrast, Urokon, Miokon, Hypaque and Renografin in the dog. The various contrast media were tested in 70 per cent solutions and it was found that Hypaque and Renografin were least toxic and Urokon most toxic, while Diodrast and Miokon occupied an intermediate position. These results were confirmed by LANCE & KILLEN (1959) in a similar investigation.

KENAN, TINDALL, MARGOLIS and WOOD (1958) investigated the effect of various procedures that might afford a protection against the lesion in the spinal cord produced by contrast medium. One ml of Urokon 70 % per kg body weight was injected into the abdominal aorta in dogs.

It was found that hypothermia in the range of 27 to 29° C gave a significant protection as did the preceding injection of procain and glucose at normal body temperature. However, they did not check whether the blood in the vessels of the spinal cord was expelled or not.

MARGOLIS *et al.* (1959) studied the role of altered circulatory dynamics when injuries were produced by Urokon to the spinal cord in dogs. The contrast medium was injected into the abdominal aorta and the circulation

was studied *in vivo* on the exposed lumbar cord. The injected solutions were as a rule labelled with a 0.5 per cent concentration of sodium fluorescein. Injection of Urokon 35 % showed an increase in the rate of the blood flow of the spinal cord after the injection, which persisted from 10 to 30 minutes in individual animals. The systemic blood flow was unaltered. Intravenous injection of contrast medium showed no effect upon the spinal cord circulation.

It was shown that a toxic dose of Urokon 70 % reached the arterial tree at its usual speed. After the pial vessels had been filled there was an arrest in the flow of the contrast medium for 40 to 120 seconds. The injected medium remained in the arterial tree as an apparently static mass for 10 to 30 seconds and then slowly disappeared from the venous bed. At the same time multiple focal extravasations of the fluorescent material developed around the pial veins.

Immediately after the injection the red cells clustered into granular masses which were more pronounced in the venous than in the arterial network. The blood flow slowed down remarkably. Vasodilatation was a prominent feature of the reaction. The circulatory status of the spinal cord rarely returned to normal. Observations up to 4 hours after the injection showed a progressive sludging and near stasis. After the experiment monolite fast blue was injected and showed incomplete filling of damaged areas in contrast with the normal ones.

WHITELEATHER & DESAUSSURE (1956) investigated the effect of contrast media in dogs, injected into the internal carotid artery, on the BBB studied with trpb. Hypaque 50 % and Diodrast 35 % produced no extravasation of trpb. In similar experiments in the dog LINDNER, CHASON, MARTIN, WEBSTER & GURDJIAN (1958) found no damage to the BBB after 4 succeeding injections of Hypaque 50 % in a total dose of 80 ml. Injection of Diodrast or Urokon produced blue-staining of the hemisphere on the injected side. Renografin gave only a slight lesion. However, in adequate amounts and concentrations the used contrast media produced an engorgement of the blood vessels, particularly in the basal ganglia, which was more pronounced after injection of Diodrast and Urokon. As the vessels were perfused with saline after death the observed engorgement would be synonymous with the stasis produced in the present experiments. This would mean that stasis could be produced by Hypaque, but the dose necessary for this is not given. HOPPE (1959) studied the effect of contrast media on the BBB in rabbits with a modification of the technique described by BROMAN & OLSSON (1948). Twentyfive ml of the solution to be tested was injected into the common carotid artery at a rate of 2 ml/sec. The lesion produced by diatrizoate sodium 50 % was of the same degree as that produced by iodopyracet 35 %. The lesion

produced by sodium acetrizoate 50 % was severer than that produced by iodopyracet 50 %. In similar studies in the rabbit VOSSE (1960) found that Urografin produced a lesion less pronounced than that corresponding to Hypaque, Miokon and Urokon.

Thus the contrast media of the diatrizoate group, i.e. Hypaque and Urografin, are the least injurious to the BBB but still capable of producing lesions.

EDSTRÖM (1961) showed that the lesion produced by Triurol in rabbits could be indicated with I^{131} -tagged human serum albumin.

The purpose of the experiments at normal body temperature was to obtain a reference series for the experiments in hypothermia. As the preliminary results showed that the lesion diminished in hypothermia, it was necessary to select such a dosage as would give lesions in almost every experiment at normal body temperature. From the investigations referred to above it seemed as if Umbradil 50 % with an application time of 20 seconds would fulfil these requirements. It was also decided that the relation between the length of the application time and the degree of the injury to the BBB produced by Umbradil 50 % should be determined.

In some of the experiments the injections were performed with ligation of the external carotid artery in order to obtain application times longer than 20 seconds. This made it possible to compare the 2 modes of injection as to the effect on certain characteristics of the injection.

A. INJECTION OF UMBRADIL INTO COMMON CAROTID ARTERY

I. UMBRADIL 50% OF 38° C

Experimental. — Ten animals were injected with Umbradil 50 % of 38° C into the left common carotid artery. The decided application time was 20 seconds. If the blood returned in the pial vessels the injection was nevertheless continued in order to test whether the injected amount or the length of the application time was the decisive factor for the degree of the obtained lesion. The brain was cut completely into about 30 sections.

Results

The experimental data are given in table 58. The decided application time of 20 seconds was achieved only in one animal. In the other animals the injection proceeded for a total of 20 seconds after the return of the blood to the pial vessels. As a rule the expelling of the blood was obtained at the beginning of the injection and then the contrast medium did not return, except in one experiment.

The expelling of blood varied in degree as sometimes only the arteries

Table 2. Normal body temperature. Injection of Umbradil 50 % of 38° C into left common carotid artery. Ranking of the various areas of vascular supply of the brain according to degree and extent of injury as judged from sections.

Rabbit No	Left internal carotid area	Right internal carotid area	Vertebral area	
			left	right
C 68	1	4	2	3
C 77	1	4	2	3
C 78	1	3	2	4
C 69	1	4	2	3
C 79	1	4	2	3
C 72	1	neg	2	neg
C 73	1	1	2	2
C 76	3	2	1	4
C 71	2	4	1	3
C 75	1	4	2	3

were filled with contrast medium. Intermittent expelling of the contrast medium occurred occasionally in the arteries and more often in the veins.

The injected volumes ranged between 25 and 40 ml with a mean of 30 ml. No obvious agreement was found between length of application time and the calculated rate of injection. The pilot injection was uneventful.

The body temperature fell about 1° C during the experiment. At the injection of Umbradil the body temperature was about 38° C. The blood pressure ranged between 40 and 90 mm Hg before the injection with a mean of about 70 mm Hg. It fell after the injection and was about 30 to 40 mm Hg towards the end of the experiment. No convulsions occurred.

Macroscopic examination. — The choroid plexuses, median eminence, hypophysis and area postrema were stained. The dura mater was blue bilaterally. The staining of the brain was as a rule uniform but could also be patchy and sometimes streaky on the cut surface. The lesion was rarely confined to the region of the injected internal carotid artery but occurred also in the other vascular areas as seen from table 2.

The injected internal carotid area was as a rule the most heavily damaged, followed by the left vertebral area, the right vertebral area and the right internal carotid area in the order given. Exceptions occurred, however, in 2 brains in which the left vertebral area was the most stained. In one of these the right internal carotid area was more damaged than the left internal carotid region. As a rule unstained areas were found within all the vascular territories. When the application time was 6 seconds the degree of the lesion tended to be less pronounced in spite of the continued injection.

The extent of the lesion did not seem dependent on the length of the application time.

Microscopic examination. — In spite of the perfusion of the vessels some trpb generally remained in the lumen of the vessels within the damaged areas. In only one brain, C 71, was the lumen of the vessels rinsed from trpb within the damaged areas. These remains of trpb within the damaged areas were also found in brains that were almost completely rinsed from trpb in the lumen of the vessels in non-damaged areas. Within the damaged areas the walls of the vessels were stained. Staining of the endothelial nuclei was a rare feature. The brain parenchyma was stained a diffuse blue, and staining of the parenchymal nuclei was often seen. Such staining was most pronounced in the cortex, hypothalamus, quadrigeminal bodies and cerebellum. When the medulla oblongata and pons were damaged, staining of the parenchymal nuclei was the rule also in these areas.

White matter such as corpus callosum, centrum ovale and internal capsule, and the white matter in the cerebellum, were as a rule stained only diffusely blue and staining of the parenchymal nuclei was an exception.

In the brain with stasis it was sometimes possible to see a staining of the endothelial nuclei in the vessel wall and the parenchymal nuclei.

In the quadrigeminal bodies the nuclei of the nerve-cells in the midline beneath the aqueduct were often stained. In the cerebellum the staining of the Purkinje cells was prominent although staining of parenchymal nuclei could also occur in the granular layer. The cerebellar nuclei located in the medullary body often showed a pronounced staining of the nerve-cells. In the cerebellum it was noted that when the lesion was faint the staining was diffuse.

The hippocampus was often stained in the cells of the ventral part, particularly the pyramidal cells. The dorsal part was fairly often stained only faintly and staining of the parenchymal nuclei was rather an exception.

The internal elastic membrane of the pericallosal artery was regularly stained.

II. UMBRADIL 50 % OF ROOM TEMPERATURE

Application time 20 seconds

Experimental. — Fourteen animals were injected with Umbradil 50 % of room temperature into the left common carotid artery. The decided application time was 20 seconds. The brain was cut completely into about 30 sections.

Results

The experimental data are given in table 59. The decided application time was achieved in 10 animals. The Umbradil solution was injected

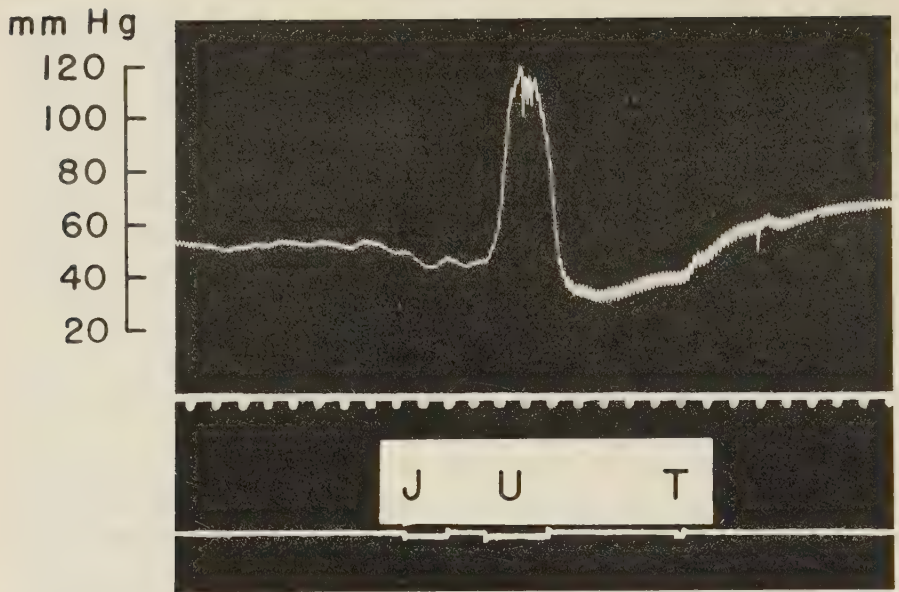


Fig. 1. Normal body temperature. Exp. C 92. Injection of Umbradil 50 % of room temperature into common carotid artery with an application time of 20 sec. Umbradil 32 ml. Inj. time 22 sec. J: Removal of jugular clamps. U: Injection of Umbradil. T: Start of infusion of trpb. The jugular clamps are replaced between U and T. Time marking: 10 sec. Note rise in blood pressure during injection.

for 20 seconds in those animals in which the blood returned during the injection. The body temperature was about 38°C at the injection of Umbradil and fell about 0.5°C towards the end of the experiment.

In about half of the experiments the expelling of the blood was not complete in all of the pial vessels. The injected volume varied from 20 to 40 ml with a mean of about 32 ml. In one experiment, C 83, the pilot injection was unsuccessful. The injection was nevertheless made and the obtained application time was only 3 seconds.

No relation was found between the calculated average injection rate and the length of application time.

The blood pressure just before the injection was on an average about 70 mm Hg and rose during the injection 25 to 70 mm Hg (fig. 1). Towards the end of the experiment the blood pressure fell to about 20 mm Hg.

In C 87 and C 91 convulsions occurred during and after the injection of Umbradil.

Macroscopic examination. — The choroid plexuses, median eminence, hypophysis and the area postrema were stained. The dura mater was

Table 3. Normal body temperature. Injection of Umbradil 50 % of room temperature into left common carotid artery with an application time of 20 seconds. Ranking of the various areas of vascular supply of the brain according to degree and extent of injury as judged from sections.

Rabbit No	Left internal carotid area	Right internal carotid area	Vertebral area	
			left	right
C 74	1	3	2	4
C 80	4	3	2	1
C 81	2	4	1	3
C 84	1	2	3	neg
C 85	1	3	2	2
C 87	1	3	2	4
C 88	1	3	2	4
C 89	1	4	2	3
C 91	1	3	2	4
C 92	1	2	3	4
C 90	1	2	3	4
C 82	1	4	2	3
C 83	1	3	2	4
C 86	2	1	neg	neg

In C 90, C 82, C 83 and C 86 the decided application time of 20 sec. was not achieved.

faintly stained or unstained on the left side in C 81, C 84, C 85, C 87, C 91, C 92, C 90, C 82 and C 83, and bilaterally in the others. The degree of the staining as judged from colour photographs and sections is given in table 59. The staining was as a rule uniform (plate I: 1,2). On the cut surface it was sometimes streaky, particularly in the cortex, or patchy and dotted. The staining could vary in degree within the same hemisphere.

The lesion generally extended over the region of the injected internal carotid artery as seen from table 3. The most heavily damaged area was generally the injected internal carotid area, followed by the left vertebral, the right internal carotid and the right vertebral areas in the order given. Exceptions occurred: In one experiment the left vertebral area was the most stained and in another the right vertebral one. In 2 experiments the right internal carotid area was more stained than the injected area. In one of them, C 86, the application time was only 3 seconds and the lesion was slight to questionable. The whole brain was damaged in C 74, C 80 and C 87. In the others, undamaged areas were regularly seen within the least damaged vascular territories. The degree of the lesion seemed to be less pronounced when the application times were 6 seconds or less.

The experiment in which the degree of staining was more pronounced in a non-injected area did not show any noticeable deviation from the others as regards blood pressure before injection, blood pressure rise during injection, injected volume, calculated average injection rate,

Table 4. *Ranking on sections of the degree of injury in 10 areas on the left side from 8 brains injected with Umbradil 50 % of room temperature into left common carotid artery with an application time of 20 seconds at normal body temperature. The areas were ranked within each animal by 2 judges. The most damaged area was given the lowest score. The sum of scores for 8 brains is given. Statistical treatment according to Friedman two-way analysis of variance.*

	Cortex	Hip- po- cam- pus	Septum pelluci- dum	White matter	Basal ganglia	Thala- mus	Hypo- thala- mus	Quadri- geminal bodies	Cere- bel- lum	Pons and medulla oblon- gata
Judge I	25.5	60	57	61.5	41	29	39	34.5	34.5	58
Judge II	32	55	59	40	55	19	53	37	31	59

Degrees of freedom=9

Judge I $\chi^2=23.3$ $P<0.01$

Judge II $\chi^2=24.2$ $P<0.01$

The Spearman rank correlation coefficient for the 2 judges=0.72. $t=2.94$. Degrees of freedom=8. $P<0.02$.

effective injection pressure (difference between injection pressure and blood pressure during injection) or injection time before expelling of the blood in the pial vessels.

In 8 brains with an application time of 20 seconds the left side of the entire brain was damaged. This was considered an opportunity to test whether a difference, if any, existed in the degree of lesion between the 10 areas of the brain given in chapter II. For this purpose the 10 areas within each brain were ranked with regard to the degree and extent of lesion. The lowest score was given to the most damaged area. The ranking was performed by 2 judges using the same criteria. The results are presented in table 4. The ranking of both judges showed that a significant difference existed from the hypothesis that the areas were damaged to the same degree. By using the Spearman rank correlation coefficient, the hypothesis that there was no association between the judges was rejected on the 2 per cent level (table 4).

The thalamus, cortex, cerebellum and the quadrigeminal bodies were among the most damaged regions, the hypothalamus, pons and medulla oblongata, basal ganglia, white matter, septum pellucidum and hippocampus among the least damaged.

Microscopic examination. — The histologic picture agreed in its main features with that seen in the series with Umbradil 50 % of body temperature. In the damaged areas trpb remained in the lumen of the vessels to a varying extent. This occurred in the stained areas also in such experiments in which one hemisphere or the cerebellum appeared unstained on macroscopic inspection. In the unstained areas trpb sometimes remained to a lesser degree in the vessels but as a rule the vessels of the undamaged areas were rinsed from trpb in the lumen.

In those areas in which the damage was slight the lesion consisted macroscopically of blue dots. Microscopically the vessel wall of these areas was stained and the vessel was surrounded by a blue area. The lumen of the vessel was always rinsed from trpb.

In the areas macroscopically more uniformly stained, the microscopic picture (plate III: 1) varied from a faint diffuse staining to a pronounced blue staining, which was as a rule accompanied by staining of the parenchymal nuclei, and sometimes stasis. Thus patches of different degrees of damage occurred within the same territory of vascular supply and within the same structure as for example the cortex.

In the areas of stasis the vessel wall and the parenchyma were as a rule unstained. Staining of the vessel wall in the areas of stasis sometimes occurred and then as a rule consisted of staining of the endothelial nuclei. Sometimes staining of the parenchymal nuclei also occurred in areas of stasis. Then the endothelial nuclei were stained and the lumen of the vessel was filled with red blood corpuscles. This may indicate that it takes some time for the stasis to develop. Areas with stained parenchymal nuclei but with unstained vessel wall were not seen. The staining of endothelial nuclei seemed to occur more often in the vicinity of areas with stasis. A few small extravasal accumulations of red blood corpuscles were exceptionally seen. The choroid plexuses were as a rule stained in the nuclei of the epithelium.

Those 8 brains that were damaged on the whole left side were studied with regard to the presence of diffuse staining of the vessel wall, staining of the endothelial nuclei, diffuse staining of the parenchyma, staining of the parenchymal nuclei and stasis. The frequency of a histologic characteristic within an area was compared with the area with the highest frequency of that particular characteristic. The results are shown in table 5. Staining of the vessel wall and diffuse staining of the parenchyma were present in all the regions studied. The differences in frequency were not significant. Staining of the endothelial nuclei was a fairly rare feature, except in the cortex. Staining of the parenchymal nuclei occurred less often in the white matter of the hemispheres and the cerebellum, the dorsal part of hippocampus, basal ganglia and thalamus than in the rhinal cortex.

A trend towards a higher frequency of the presence of staining of the parenchymal nuclei in areas macroscopically more intensely stained appeared when these results were compared with those obtained on ranking the various regions macroscopically according to degree and extent of injury. An exception was, however, the thalamus which in spite of a high ranking macroscopically showed staining of the parenchymal nuclei in only a few brains. Stasis of the thalamus and hypothalamus occurred

Table 5. *The frequency of brains with various histologic characteristics in different areas on the left side. The material consisted of 8 brains damaged on the whole left side in the series with injection of Umbradil 50 % of room temperature into left common carotid artery with an application time of 20 seconds at normal body temperature.*

Area	Staining of vessel wall		Parenchymal staining		Stasis	Number of brains with injury to the area	Number of brains with stasis only
	Diffuse	Endothelial cell nuclei	Diffuse	Nuclei			
Cortex paras.	7	4	7	5	4	8	1
Cortex convex.	7	5	7	7	6	8	1
Rhinal cortex	8	5	8	8	3	8	0
Hippocampus dorsal	8	0 ¹	7	2 ²	0 ²	8	0
ventral	7	1	7	6	2	8	1
Sept. pell.	8	1	8	7	2	8	0
Corp. call.	7	0 ¹	4	2 ²	0 ²	4	0
Centr. ovale	4	1	2	1 ¹	2	4	2
Caps. int.	8	1	6	0 ³	2	6	0
Comiss. ant.	7	1	6	0 ³	2	6	0
N. lentiformis	7	1	7	2 ¹	2	8	1
N. caudatus	7	3	7	2 ¹	3	8	1
Thalamus	8	2	8	3 ¹	1 ¹	8	0
Hypothalamus	8	2	8	8	1 ¹	8	0
Quadr. bodies	8	2	8	8	2	8	0
Cerebellum	8	1	8	7	2	8	0
Medullary laminae	5	0	4	0 ²	1 ¹	5	1
Medullary body	7	0	7	1 ²	1 ¹	8	1
Cerebellar nuclei	7	0	7	5	1 ¹	8	1
Pons and med. obl.	6	0 ¹	6	6	0 ²	6	0
Oliva superior	5	0 ¹	5	4	0 ²	5	0

Statistical treatment with Fisher's exact probability test for 2×2 contingency tables within each group of histologic characteristics. The area with the highest incidence of damage within each group was the reference area. This was the rhinal cortex except in stasis where cortex convex. was the reference area. In areas with stasis only, staining of the vessel wall and of the parenchyma usually did not occur. The number of brains with stasis only has therefore been added to the figures in these columns in the statistical treatment.

¹ $P < 0.05$ ² $P < 0.01$ ³ $P < 0.001$.

less often than in the convexity of the cortex, which also applies to the corpus callosum and the dorsal part of the hippocampus. Stasis was also relatively rare in the structures in the posterior fossa.

Application time 10 seconds

Experimental. — Six animals were used. Umbradil 50 % of room temperature was injected into the left common carotid artery with the animal's head turned to the left. The application time was 10 seconds. After fixation the brain was cut, 3 sections being made from 4 parts of the brain.

Results

The experimental data are given in table 60. The body temperature at the injection had a mean of 37.6°C . The predetermined application time was achieved in 5 animals. In one animal no application time was achieved. In some of the 5 animals with an application time of 10 seconds the expelling of blood was intermittent in the veins but complete in the arteries. In the experiment in which no application time was achieved the pilot injection did not expel the blood in the veins. The blood in the pial vessels returned about 3 seconds after the end of injection. The injected volume varied from 11 to 35 ml.

The blood pressure before the injection varied from 50 to 110 mm Hg with a mean of 90 mm Hg. The maximal rise in blood pressure during injection varied from 4 to 84 mm Hg with a mean of 28 mm Hg. The smaller rise occurred in those animals which had a blood pressure above 100 mm Hg before the injection. Towards the end of the experiments the blood pressure fell to a mean of about 40 mm Hg.

In 2 animals, C 273 and C 277, convulsions occurred just after the injection. The trpb was infused in about 10 minutes.

Macroscopic examination. — The areas normally permeable to trpb including the supraoptic crest were stained. The dura mater was faintly stained in C 277 and in C 273 more stained on the right side. In the others the dura mater was stained bilaterally. The degree of injury as judged from colour photographs and sections is given in table 60. The staining on the surface of the brain was mostly uniform even though the intensity could vary. In C 281 the staining was faint and appeared as fairly large dots with more intense staining within the left prosencephalon.

On the cut surface the staining was as a rule diffuse. The white matter and the dorsal part of the hippocampus appeared less intensely stained. The basal ganglia, thalamus, and hypothalamus were as a rule stained to the same degree as the cortex. In C 277, stasis occurred in the whole left half of the prosencephalon. The stasis also occurred on the right side in the basal ganglia, thalamus and hypothalamus. On the left side stasis also occurred in the quadrigeminal bodies and the cerebellum. In C 271 the left olfactory bulb was stained.

The distribution of the lesion with regard to the different vascular areas is seen from table 6. The brain with none or only faint staining of the region of the internal carotid artery on the side of injection showed almost no rise of the blood pressure during the injection. The dura mater was faintly stained on the left side in that experiment. The 2 experiments with an unexpected location of the lesion did not show any tendency to differ from the experiments with an expected location of the lesion as

Table 6. *Normal body temperature. Injection of Umbradil 50 % of room temperature into left common carotid artery with an application time of 10 seconds. Ranking of the various areas of vascular supply of the brain according to degree and extent of injury as judged from sections.*

Rabbit No	Left internal carotid area	Right internal carotid area	Vertebral area	
			left	right
C 269	1	3	2	neg
C 273	4	1	3	2
C 275	2	4	1	3
C 277	1	3	2	4
C 281	1	neg	neg	neg

regards length of the injection period before the expelling of blood in the pial vessels, length of time for refilling of pial vessels with blood after the end of the injection, calculated average injection rate and the calculated effective injection pressure.

Microscopic examination. — The microscopic picture was essentially the same as in the series with an application time of 20 seconds. In this series, too, it was observed that absence of trpb in the lumen of the vessels in an undamaged part of the brain did not necessarily mean that the vessels in the damaged parts were completely rinsed.

In C 273 the parenchyma of the left hemisphere was not stained and the vessels were not completely rinsed. The vessel wall was occasionally stained. The brain (C 281) with faint staining showed diffuse staining of the parenchyma, but none of the parenchymal nuclei. The wall of the pericallosal arteries was stained diffusely. The vessel wall and the epithelium of the choroid plexuses were as a rule stained, occasionally with staining of the nuclei.

Application time 5 seconds

Experimental. — Five animals were used. Umbradil 50 % of room temperature was injected into the left common carotid artery with the animal's head turned to the left. The application time was 5 seconds. The brain was cut, 3 sections being made from 4 parts of the brain.

Results

The experimental data are given in table 61. The body temperature at the injection was on an average 37.8° C. The decided application time was achieved in all animals. The blood in the pial vessels was completely expelled. The injected volume was about 14 ml. The pial vessels refilled with blood about 3 seconds after the end of injection.

Table 7. *Normal body temperature. Injection of Umbradil 50 % of room temperature into left common carotid artery with an application time of 5 seconds. Ranking of the various areas of vascular supply of the brain according to degree and extent of injury as judged from sections.*

Rabbit No	Left internal carotid area	Right internal carotid area	Vertebral area	
			left	right
C 283	1	4	2	3
C 285	1	4	2	2
C 287	4	3	1	2
C 289	1	neg	2	neg
C 292	3	4	1	2

The blood pressure before the injection was on an average of about 90 mm Hg. The maximal rise of blood pressure during the injection from the pre-injection level varied from 20 to 44 mm Hg with a mean of 30 mm Hg. The increase tended to be higher in the animals with a lower blood pressure before the injection. The fall in blood pressure towards the end of the experiments was fairly small.

In C 283 and C 285 convulsions occurred immediately after the end of the injection. The trpb solution was infused in about 8 minutes.

Macroscopic examination. — The areas normally permeable to trpb including the supraoptic crest were stained. In C 285 the dura mater was more intensely stained on the right side. In the others the dura mater was stained bilaterally. The degree of injury as judged from colour photographs and sections is given in table 61. The staining was as a rule uniform. It could vary in intensity within one and the same vascular territory. In 2 brains the staining was dotty and patchy in some areas. On the cut surface streakiness was occasionally seen in the cortex and patchiness in the pons and medulla oblongata and the cerebellum.

The distribution of the lesion with regard to different vascular territories is seen from table 7. In 2 brains the staining was more pronounced in the vertebral area than in the injected area. The calculated effective injection pressure was somewhat higher in one of them. The other data of the injection procedures, as injection time before expelling of blood, the time for refilling of pial vessels with blood after the end of the injection, blood pressure increase during the injection, the amount injected and the calculated average injection rate, did not show any tendency to deviation from the other experiments. The brain with fainter staining of the dura mater on the left side showed an expected location of the lesion.

Microscopic examination. — The microscopic picture was mainly of the same appearance as in the before-mentioned series. The rinsing was as a rule complete within the unstained areas even if occasionally vessels

were seen with trpb in the lumen. Within the damaged areas the vessel wall was stained and it occurred that trpb remained in the lumen.

In the macroscopically blue-stained areas, the vessels were surrounded by a diffuse blue staining, as a rule coalescing, but occasionally confined to the vicinity of the vessels. Generally, staining of the parenchymal nuclei occurred more often and more pronouncedly in the areas that were macroscopically more intensely stained. The staining of the parenchymal nuclei did not seem to be as frequent as in the series with an application time of 20 seconds.

B. INJECTION INTO COMMON CAROTID ARTERY WITH LIGATION OF EXTERNAL CAROTID ARTERY

An application time of 20 seconds on injection into the common carotid artery requires a volume of the injected solution of up to 40 ml. To obtain substantially longer application times with this mode of injection it is necessary to change syringes or use syringes holding 100 ml. None of these procedures is particularly feasible. On ligation of the external carotid artery the amount required is considerably smaller (STEINWALL 1958).

In order to obtain an application time of 40 seconds, experiments with ligation of the external carotid artery were performed. In order to check whether any major differences in the appearance of the lesion existed with this mode of injection, a few experiments with an application time of 20 seconds were also performed. To make possible a comparison between the 2 modes of injection it was decided not to change the technique in other details. Umbradil of 38° C was used to test the effect of the ligation on the possibility of obtaining the decided application time when the contrast medium was of body temperature.

I. UMBRADIL 50 % OF 38° C

Application time 40 seconds

Experimental. — Five animals were used. Umbradil 50 % of 38° C was injected into the left common carotid artery after ligation of the external carotid artery. The application time was 40 seconds. The brain was cut, 3 sections being made from 4 parts of the brain.

Results

The experimental data are given in table 62. The body temperature at the time of injection was about 37.5° C. The decided application time

Table 8. *Normal body temperature. Injection of Umbradil 50 % of 38° C into left common carotid artery with ligation of external carotid artery with an application time of 40 seconds. Ranking of the various areas of vascular supply of the brain according to degree and extent of injury as judged from sections.*

Rabbit No	Left internal carotid area	Right internal carotid area	Vertebral area	
			left	right
C 186	4	3	2	1
C 188	4	1	3	2
C 190	3	4	2	1
C 192	1	1	4	3
C 194	2	neg	1	3

of 40 seconds was achieved in all animals. In C 194 the blood in the vessels in the fore part of the opening of the skull was not expelled. The injected volume was about 9 ml. The pial vessels refilled with blood about 5 seconds after the end of the injection.

The blood pressure fell from 0 to 40 mm Hg when the clamps on the jugular veins were removed. The rise during the injection was 20 mm Hg in 2 experiments, in one no rise occurred, and in the 4th the rise did not reach the pre-injection level. After the injection the blood pressure generally fell and rose during the injection of the trpb solution and then fell again towards the end of the experiment. Convulsions occurred in 4 of the animals, C 194 being an exception.

Macroscopic examination. — The areas normally permeable to trpb were stained in all brains except the hypophysis, which was not stained in one experiment. The dura mater was stained in all experiments but only faintly in C 186, C 188 and C 192. The degree of injury as judged from colour photographs and sections is given in table 62. The staining was generally uniform. The degree, however, varied within the same vascular area of the brain. Stasis could be found in part of a hemisphere. Occasionally the staining was dotty or patchy. In C 194 part of the surface of the left hemisphere was almost undamaged. The location of the area was such that it might represent the part of the brain in which the blood in the pial vessels was not expelled during the injection. On the cut surface the staining was streaky or patchy in areas faintly stained.

The distribution of the lesion with regard to different vascular territories is seen from table 8. The difference in damage between the various regions was as a rule slight, exceptions being C 190 with a more intense staining of the vertebral area and C 194 with an almost negative right internal carotid area. The injection characteristics of C 192 with an expected location of the lesion did not differ appreciably from those of the others. In C 194, with an almost unstained right side, the time for refilling

of the pial vessels with blood after the end of the injection was the shortest one recorded. This was also the smallest volume injected.

Microscopic examination. — The microscopic picture agreed in its principal features with that described for the other series with injection of Umbradil. Thus staining of the vessel wall, diffuse staining of the parenchymal nuclei and stasis occurred. Staining of the nuclei of the endothelial cells was common also in the areas that were not located in the vicinity of areas with stasis.

The occurrence of staining of the parenchymal nuclei seemed to be more frequent than in for instance the series with injection into the common carotid artery with an application time of 5 seconds.

The rinsing of the vessels was fairly good. In C 194, in which several parts of the brain were unstained, trpb was seen in the lumen of the vessels in the macroscopically unstained areas, particularly in the cortex. Occasionally staining of the vessel wall without trpb in the parenchyma was seen in these areas. However, the pons and medulla oblongata, which were also unstained, were completely rinsed.

Stasis was found in experiments C 188 and C 192 and in small areas in C 186 and C 190. In C 190 stasis occurred only in a small parasagittal area but in C 186 on the right side in the pyriform lobe, the hypothalamus and the cerebellum and on both sides in the superior olivary nucleus and its immediate vicinity.

Application time 20 seconds

Experimental. — Three animals were used. Umbradil 50 % of 38° C was injected into the left common carotid artery after ligation of the external carotid artery. The application time was 20 seconds. The brain was cut, 3 sections being made from 4 parts of the brain.

Results

The experimental data are given in table 63. The body temperature at the time of the injection was about 38° C. The decided application time, 20 seconds, was achieved. The injected volume varied from 4—5 ml. The pial vessels were refilled with blood about 3 seconds after the end of the injection.

The blood pressure before the injection varied from 80 to 95 mm Hg. As a rule it fell some 10 to 20 mm Hg at the opening of the jugular veins. The rise in the blood pressure was as a rule low but in C 171 an increase of 45 mm Hg occurred after the injection. With the exception of this experiment the mean rise was 5 mm Hg. After the end of the injection the blood pressure was generally low, about 40 mm Hg. The trpb solu-

Table 9. *Normal body temperature. Injection of Umbradil 50 % into left common carotid artery with ligation of external carotid artery with an application time of 20 seconds. Ranking of the various areas of vascular supply of the brain according to degree and extent of injury as judged from sections.*

Rabbit No	Left internal carotid area	Right internal carotid area	Vertebral area	
			left	right
C 171	3	4	2	1
C 175	4	2	3	1
C 180	4	1	2	3

tion was infused in about 3—6 minutes. Convulsions occurred in 2 of the experiments (C 171, C 175).

Macroscopic examination. — The areas normally permeable to trpb were stained. The dura mater was stained in all experiments but only faintly in C 171. The degree of injury as judged from colour photographs and sections is given in table 63. The staining was fairly uniform even if variations in the intensity occurred within the same vascular area of the brain. The staining was sometimes dotted or patchy as in the left hemisphere in C 175.

The distribution of the lesion with regard to different vascular territories is seen from table 9. The lesion was thus most pronounced either in the right vertebral area or in the right internal carotid area. The experimental data do not show any obvious difference referable to the distribution of the lesion.

Microscopic examination. — The microscopic picture had the same main appearance as in the other series with Umbradil 50 % at normal body temperature. Staining of the endothelial nuclei was, however, very rare. The nuclei of the parenchyma was often stained in regions with intense macroscopic staining. The vessels in unstained regions were as a rule well rinsed. In some vessels, however, trpb sometimes remained in the vessel and occasional staining of the vessel wall was also seen. The vessels within damaged regions were not always completely rinsed. Particularly in C 180 trpb remained in the lumen of the vessels in the damaged regions in spite of the fact that the vessels in the undamaged regions were fairly well rinsed.

C. INJECTION OF UMBRADIL 50% WITH REGARD TO EXPERIMENTS PERFORMED IN HYPOTHERMIA

The blood pressure is low in hypothermic animals. In order to evaluate the significance of the low blood pressure for the genesis of the injury to the BBB, experiments were performed on animals of normal body

temperature with a lowered blood pressure. The level of the blood pressure might play a part either in the genesis of the lesion or for the penetration of the indicator substance, trpb, from the blood to the brain parenchyma. If the experiments showed that the damage of the BBB was prevented by lowering the blood pressure, no information would be gained as to which of the 2 above-mentioned factors is the active one. If the lesion was not prevented it would mean that the low blood pressure had no effect on either of these 2 factors.

Further it was found desirable to study the effect of cooled Umbradil on the injury to the BBB in order to ascertain whether the effect seen in hypothermia was bound to the temperature of the animal or of the contrast medium.

I. INJECTION INTO COMMON CAROTID ARTERY AFTER REDUCTION IN BLOOD PRESSURE BY MEANS OF HEXAMETHONIUM BROMIDE

Experimental. — Five animals were used. Hexamethonium bromide (Vegolysen, May & Baker, Dagenham, England) was administered intravenously in a dose of 4 mg/kg body weight. Umbradil 50 % of room temperature was then injected into the left common carotid artery with a predetermined application time of 20 seconds. The brain was cut, 3 sections being made from 4 parts of the brain.

Results

The experimental data are given in table 64. The body temperature at the injection had a mean of 38.2° C. The decided application time was achieved except in one animal with an application time of 12 seconds in spite of the injection pressure being increased to a maximum of 250 mm Hg. The injected volume was about 30 ml. The blood pressure fell about 35 mm Hg on injection of hexamethonium bromide and was about 40 mm Hg before the injection of Umbradil. The increase in blood pressure during the injection was fairly low with a mean of about 15 mm Hg (range 5—30 mm Hg), (fig 2).

The trpb solution was infused in about 5 minutes. The blood pressure level after the injection was almost about 40 mm Hg with the exception of C 107, in which the blood pressure level rose to about 100 mm Hg during the infusion of trpb.

Macroscopic examination. — The areas normally permeable to trpb were stained blue. In 4 experiments the lesion was more pronounced in the right side of the cerebrum or in the cerebellum and in these animals the dura mater on the left side was faintly stained.

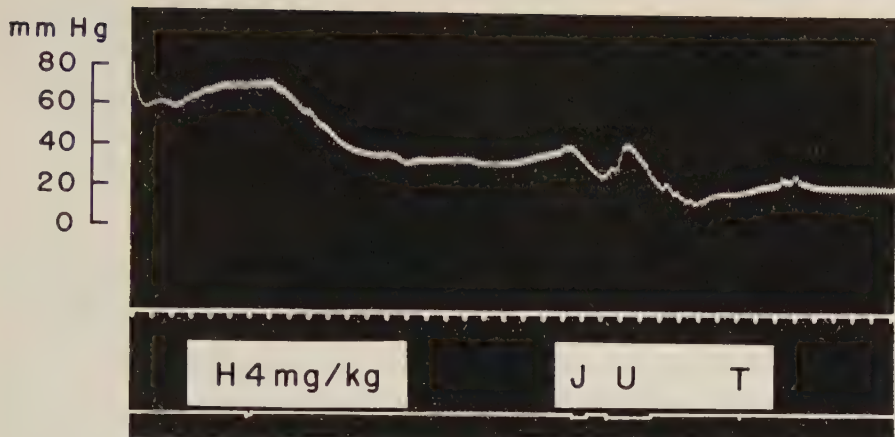


Fig. 2. Normal body temperature. Exp. C 106. Injection of Umbradil 50 % of room temperature into common carotid artery with an application time of 20 sec. Reduction in blood pressure by means of hexamethonium bromide. Umbradil 36 ml. Inj. time 23 sec. H: Intravenous inj. of hexamethonium bromide 4 mg/kg body wt. J: Removal of jugular clamps. U: Injection of Umbradil. T: Start of infusion of trpb. The jugular clamps are replaced between U and T. Time marking: 10 sec. Note virtual absence of blood pressure rise above pre-injection level.

The degree of injury on sections and colour photographs is given in table 64. The macroscopic appearance was like that described for Umbradil 50 % of room temperature. The distribution of the lesion between different areas of vascular supply is given in table 10. Thus the lesion was as a rule most pronounced on the side opposite to the injection.

Microscopic examination. — The principal features as described before were also present in this series. Thus there was staining of the vessel wall occasionally with staining of the endothelial nuclei, diffuse staining of the parenchyma, staining of the parenchymal nuclei and stasis. In those

Table 10. Normal body temperature. Injection of Umbradil 50 % of room temperature into left common carotid artery in animals treated with hexamethonium bromide. Ranking of the various areas of vascular supply of the brain according to degree and extent of injury as judged from sections.

Rabbit No	Left internal carotid area	Right internal carotid area	Vertebral area	
			left	right
C 106	4	1	3	2
C 107	4	1	3	2
C 108	4	1	3	2
C 109	1	2	3	neg
C 111	4	2	3	1

animals in which the injected side was less intensely stained than the right side or the cerebellum, diffuse staining of the vessel wall was as a rule seen with none or only faint staining of the surrounding parenchyma.

II. INJECTION OF COOLED UMBRADIL 50 % INTO COMMON CAROTID ARTERY

Experimental. — The Umbradil solution was cooled in the ampoules in running tap water of a temperature of 11 to 12° C for 2 to 5 hours. The syringe was cooled in tap water before aspiration of the solution. The Umbradil was then injected immediately. Five animals were used. The animal's head was turned to the left during the injection into the left common carotid artery. The brain was cut, 3 sections being made from 4 parts of the brain.

Results

The experimental data are given in table 65. The body temperature at the injection had a mean of 36.8° C. The injected volume was about 32 ml. The decided application time was achieved in 4 of the experiments. In one animal it was 4 seconds. The injection was continued for 16 additional seconds. The time for refilling of the pial vessels with blood was about 3 seconds with the exception of one animal in which it was 12 seconds. The blood pressure before the injection varied from 55 to 115 mm Hg. The rise in blood pressure during the injection varied between 25 and 60 mm Hg with a mean of 37 mm Hg.

The trpb solution was infused in about 7 minutes. One of the animals (C 296) died before the infusion of trpb was started.

In one animal the brain temperature on the injected side was measured during the injection. It fell from 36.4° C to a minimum of 35.2° C during the injection.

Macroscopic examination. — The areas normally permeable to trpb including the supraoptic crest were stained blue with the exception of C 296. The dura mater was less intensely stained on the left side in C 293, C 295, C 297. In C 296 the dura mater was not stained. The macroscopic appearance of the lesion was in agreement with that described for Umbradil 50 % of room temperature with an application time of 20 seconds. The degree of injury on sections and on colour photographs is given in table 65. In C 294 with the short application time the area of the convexity of the hemispheres corresponding to the burr hole was only faintly stained. In C 296 stasis occurred in the left side of the thalamus, the quadrigeminal bodies and the cerebellum.

The distribution of the lesion between different areas of vascular

Table 11. Normal body temperature. Injection of cooled Umbradil 50 % into left common carotid artery. Ranking of the various areas of vascular supply of the brain according to degree and extent of injury as judged from sections.

Rabbit No	Left internal carotid area	Right internal carotid area	Vertebral area	
			left	right
C 293	4	3	1	2
C 294	1	2	4	3
C 295	4	3	2	1
C 297 ¹	neg	neg	1	2
C 296	neg	neg	1	2

¹ According to microscopic investigation.

supply is given in table 11. Thus the lesion was most pronounced in the area of the vertebral artery in 4 experiments. In 2 of these (C 293 and C 295) the left internal carotid area was faintly stained with negative areas intervening between areas with streaky staining. The area of the pericallosal artery on the left side was not very intensely stained in these 2 experiments. The animal with an expected location of the lesion had the shortest application time. Otherwise no differences were found in the injection characteristics between the animals. In C 297 the staining was of faint intensity in all areas and it was only on microscopic examination that it was possible to distinguish between the areas.

Microscopic examination. — The microscopic characteristics were the same as in the series with Umbradil 50 % of room temperature. The left internal carotid area in C 293 and C 295 showed staining of the vessel wall of a diffuse type. Only exceptionally did staining of the endothelial nuclei occur and then in larger vessels, mostly with packed red blood corpuscles in the lumen. In C 295 minute staining around the vessels was seen fairly frequently. In C 297, which was macroscopically faintly stained, a small area of stasis in the vermis cerebelli was seen. Faint, diffuse parenchymal staining was also seen in the cerebellum. In the thalamus, faint staining of the parenchyma was seen. In other parts of the brain the vessels were poorly rinsed but without any unmistakable parenchymal staining.

D. COMPARISON OF SERIES

In an attempt to analyse the effect of the mode of injection on the various injection data and to analyse the factors influencing the localisation and degree of injury, the data from the different series were compared.

Table 12. Normal body temperature. The degree of injury produced by Umbradil 50 %. Injury rated between 2 classes has been accounted for in the higher class. The figures denote number of animals.

Experimental series	Degree of injury on sections				
	++++	+++	++	+	neg
Injection into common carotid artery Umbradil of 38° C inj. for at least 20 sec					
Appl. time 20—16 sec	1	1	1	0	0
" " 12—9 sec	0	3	2	0	0
" " 6 sec	0	0	0	2	0
Umbradil of room temp. inj. for at least 20 sec					
Appl. time 20 sec	4	6	0	0	0
" " 12 sec	0	1	0	0	0
" " 6 sec	0	0	1	0	0
" " 3 sec	0	0	0	2	0
Umbradil of room temp. Appl. time 10 sec	1	2	1	1	0
" " 5 sec	0	3	2	0	0
Injection with ligation of external carotid artery Umbradil of 38° C					
Appl. time 40 sec	2	2	1	0	0
" " 20 sec	1	2	0	0	0
Treatment with hexamethonium bromide					
Appl. time 20 sec	0	3	1	0	0
" " 12 sec	0	1	0	0	0
Cooled Umbradil					
Appl. time 20 sec	3	0	0	1	0
" " 4 sec	0	1	0	0	0

In table 12 the degree of injury on the cut surface in the various series is summarized. With decreasing application time the frequency of less damaged brains increased and this seemed to be independent of whether the injection was continued or not, after the pial vessels had been refilled with blood. Reduction of the blood pressure with hexamethonium bromide and cooling of the Umbradil had no virtual effect on the degree of the impairment of the BBB.

Of the 52 experiments reviewed, the lesion was nowhere exclusively located in the internal carotid area on the same side as the injection. The areas of the vertebral arteries and of the internal carotid artery on the side opposite to the injection were often damaged too. It might be expected that the lesion would be most pronounced in the area of the internal carotid artery on the side of the injection. However, as seen from table 13, this was not always the case. Out of the 52 experiments, 28 had the most pronounced lesion within the expected area. The other 24 showed an

Table 13. *Normal body temperature. Distribution of injury between different areas of vascular supply at injection of Umbradil 50 %. The figures denote number of animals.*

Experimental series	Left internal carotid area most damaged or same as others	Left internal carotid area damaged		Left internal carotid area negative or slightly damaged	
		Right internal carotid area most damaged	Vertebral area most damaged	Right internal carotid area most damaged	Vertebral area most damaged
Injection into common carotid artery Umbradil of 38° C inj. for at least 20 sec Appl. time 20—16 sec " " 12— 9 sec " " 6 sec	3	0	0	0	0
	4	0	1	0	0
	1	0	1	0	0
Umbradil of room temp. inj. for at least 20 sec Appl. time 20 sec " " 12 sec " " 6 sec " " 3 sec	8	0	2	0	0
	1	0	0	0	0
	1	0	0	0	0
	1	0	0	1	0
Umbradil of room temp. Appl. time 10 sec " " 5 sec	3	0	1	1	0
	3	0	0	0	2
Injection with ligation of external carotid artery Umbradil of 38° C Appl. time 40 sec " " 20 sec	1	1	2	0	1
	0	1	1	1	0
Treatment with hexamethonium bromide Appl. time 20 sec " " 12 sec	0	0	0	3	1
	1	0	0	0	0
Cooled Umbradil Appl. time 20 sec " " 4 sec	0	0	0	0	4
	1	0	0	0	0
Total	28	2	8	6	8

Table 14. *Normal body temperature. Length of obtained application time at injection of Umbradil 50 % of room and body temperature with a decided application time of 20 seconds into common carotid artery. Statistical treatment according to Fisher's exact probability test. The figures denote number of animals.*

	Appl. time 19—20 sec	Appl. time <16 sec
Umbradil of room temp.	10	4
Umbradil of body temp.	2	8

$P=0.036$

unexpected location of the maximal lesion. In 10 out of these the left internal carotid area was also damaged and in 14 this area was almost undamaged. Ligation of the external carotid artery did not diminish the frequency of an unexpected location of the lesion. In the 2 series with injection of Umbradil with an application time of 10 and 5 seconds, respectively, the animal's head was turned to the left during the injection. Nor did this procedure diminish the frequency of an unexpected location of the lesion. On injection of cooled Umbradil, only one brain had the most pronounced lesion on the side of the injection. In this series too, the animal's head was turned to the left during the injection. In the series with decreased blood pressure owing to injection of hexamethonium bromide, most of the lesions had an unexpected location.

The decided application time was difficult to obtain with Umbradil 50 % of body temperature on injection into the common carotid artery. Umbradil 50 % of room temperature was more successful in this respect. As shown in table 14 the predetermined application time of 20 seconds was more frequently obtained by Umbradil 50 % of room temperature than of body temperature. The difference in frequency was almost significant.

However, at injection of Umbradil of body temperature with ligation of the external carotid artery the decided application time was achieved in a total of 8 experiments, even with an application time of 40 seconds. At injection of Umbradil of room temperature into the common carotid artery with application times of 10 and 5 seconds, the decided application time was obtained in 10 out of 11 experiments. In the 2 series with reduced blood pressure and cooled Umbradil, the decided application time was achieved in 8 out of 10 experiments.

In the experiments where the decided application time was achieved, incomplete expelling as intermittent expelling of the blood occurred, occasionally with expelling in the arteries only during part of the injection. The results from the various series with injection of Umbradil are summarized in table 15. The frequency of incomplete expelling seemed to

Table 15. *Normal body temperature. Number of animals with complete and incomplete expelling of blood in pial vessels during injection of Umbradil 50 %, when the decided application time was achieved.*

Degree of expelling	Injection into common carotid artery					Injection with ligation of external carotid artery	
	Umbradil of room temperature				Cooled Umbradil	Umbradil of body temp.	
	Application time sec			Hexamethonium bromide		Application time sec	
	20	10	5			40	20
Complete	5	2	5	3	4	3	3
Incomplete	5	3	0	1	0	2	0

In the series denoted as hexamethonium bromide and cooled Umbradil, respectively, one experiment in each series, in which the decided application time was not achieved, is excluded.

be higher in the experiments with injection into the common carotid artery than at injection with ligation of the external carotid artery. However, the experiments with the latter mode of injection are too few to admit of any definite conclusions with regard to the effect of the ligation of the external carotid artery on the degree of expelling of the blood in the pial vessels.

The time that elapsed after the start of injection until the blood in the pial vessels was expelled was of about the same magnitude, 2—4 seconds, in both modes of injection.

The relation between the length of application time and the mean volume of injected Umbradil is presented in fig. 3. It is seen that the mean volume is proportional to the length of the application time at injection into the common carotid artery. At injection with ligation of the external carotid artery the mean volume was about twice as large when the application time was doubled.

In table 16 is shown that the difference between the mean volumes was significant at injection of Umbradil of room temperature into the common carotid artery with the application times 20 and 10 seconds. The difference between the mean volumes for the application times 10 and 5 seconds was not significant. At injection into the common carotid artery with ligation of the external carotid artery of Umbradil 50 % of body temperature the difference between the mean volumes for the application times 20 and 40 seconds was significant on the 2 per cent level.

The data for the blood pressure rise during the injection are summarized in table 17, including the values obtained at injection of saline of normal body temperature and an otherwise unpublished series with injection of Umbradil 50 % of body temperature into the common carotid artery for

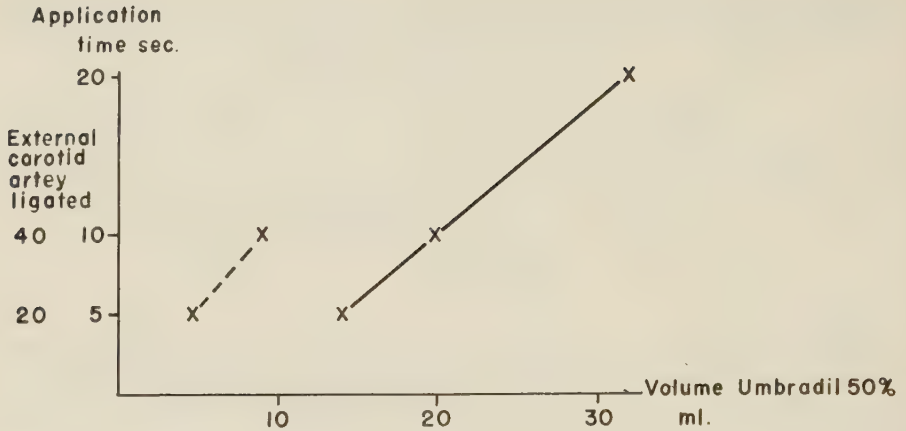


Fig. 3. Relation between mean volumes of Umbradil 50 % and application time at normal body temperature.

- Injection into common carotid artery of Umbradil of room temperature.
 - - - Injection into common carotid artery with ligation of external carotid artery of Umbradil of 38° C.

15 seconds. In this latter series the decided application time was not regularly achieved. As seen from tables 17 and 18 the blood pressure rise at injection into the common carotid artery tended strongly to be higher at injection of Umbradil than of saline. Both Umbradil cooled to about 12° C and Umbradil of body temperature induced a rise of the blood pressure of about the same magnitude as that of room temperature.

On injection of Umbradil of room temperature there seemed to be a tendency for the blood pressure rise to be lower with the shorter applica-

Table 16. Normal body temperature. Volume of Umbradil 50 % of room temperature injected into common carotid artery and volume of Umbradil 50 % of body temperature injected with ligation of external carotid artery. Statistical treatment with *t*-test. Figures in brackets denote number of animals.

Application time	Volume ml $\pm$ S.D.	Difference $\pm$ S.E.	Degrees of freedom	P
Injection into common carotid artery				
20 sec (10)	31.9 $\pm$ 6.2	12.3 $\pm$ 3.96 5.4 $\pm$ 4.17	13	<0.01
10 sec (5)	19.6 $\pm$ 9.2		8	>0.05
5 sec (5)	14.2 $\pm$ 1.7			
Injection with ligation of external carotid artery				
40 sec (5)	9.0 $\pm$ 2.0	4.3 $\pm$ 1.22	6	<0.02
20 sec (3)	4.7 $\pm$ 0.6			

Table 17. Normal body temperature. Blood pressure rise in mm Hg during injection of Umbradil and saline.

	Injection into common carotid artery										Injection with ligation of external carotid artery			
	Umbradil of room temperature						Cooled Umbradil	Umbradil of body temp.	Saline of room temp.	Saline of body temp.	Umbradil of body temp.		Saline of body temp.	
	Application time sec				Hexamethonium bromide	Application time sec					Application time sec			
	20	10	5											
	Mean Range	40	28	30	14	37	35	11	9	8	3	22	4	
		24-68	4-84	20-44	5-30	14-60	0-50	-10-30	4-14	-8-20	0-10	6-36		
Number of animals	6	5	5	5	5	5	11	4	4	3	3	1		

The data for the series 'Umbradil of body temp.' are otherwise unpublished

Table 18. *Statistical treatment of the data in table 17.*

Kruskal-Wallis test			
Application time and blood pressure increase during injection of Umbradil 50 % of room temperature into common carotid artery			
Application time sec	20	10	5
Number of animals	6	5	5
Sum of ranks	63.5	31.5	41
Mean of ranks	10.6	6.3	8.2

$H=2.2$ Degrees of freedom=2 $P>0.05$

Mann-Whitney U test		
Subject of test	U	P
Injection of Umbradil into common carotid artery		
Appl. time 20 sec versus appl. time 10 sec	8.5	>0.05
Appl. time 20 sec versus group treated with hexameth. brom.	1	<0.01
Injection into common carotid artery		
Inj. saline room temp. versus inj. Umbradil appl. time 20 sec	2.5	<0.002
" " " " " " " " " " 10 sec	18.5	>0.05
" " " " " " " " " " 5 sec	7.5	<0.05
Inj. saline body temp. versus Umbradil of body temp.	4	>0.05

tion times. This tendency was not significant either on comparison of the 3 groups with Kruskal-Wallis one-way analysis of variance or on testing the 2 groups with the most pronounced difference in blood pressure rise with the Mann-Whitney U test (table 18). Furthermore the blood pressure rise was obtained also at injection into the common carotid artery when the application time was very short or even absent (see tables 59 and 60).

At injection into the common carotid artery of Umbradil of room temperature the blood pressure rise was lower in animals treated with hexamethonium bromide. On comparison of this series with the experiments with the corresponding application times presented in table 59, the difference was found to be significant (table 18).

The blood pressure rise was definitely lower at injection of Umbradil into the common artery with ligation of the external carotid artery. At injection of saline the blood pressure rise with this mode of injection was of about the same magnitude as that for Umbradil.

There was no general tendency for a relation between the amount of injected Umbradil and the height of blood pressure rise within the series.

In some of the experimental series the time for the refilling of the pial vessels with blood after the end of the injection was recorded. The

Table 19. *Staining of dura mater at normal body temperature. The figures denote number of animals.*

Experimental series	Not stained	Faintly stained	Patchy staining	Staining most pronounced on side opposite to injection	Bilateral staining
Injection of saline into common carotid artery					
rinsing with saline	0	1	0	1	6
rinsing with formalin	0	0	0	0	3
no rinsing	0	0	0	0	3
with ligation of external carotid artery	0	0	0	1	3
Injection of air in saline into common carotid artery	0	0	0	0	5
with ligation of external carotid artery	0	0	0	0	3
Rinsing with air in saline	0	0	0	0	2
Injection of Umbradil 50 % of room temperature into common carotid artery					
Appl. time 20 sec	0	0	0	9	5
Appl. time 10 sec	0	1	0	1	4
Appl. time 5 sec	0	0	0	1	4
Injection of hexamethonium bromide	0	0	0	3	2
Umbradil cooled before inj.	1	0	0	3	1
Umbradil of 38° C.	0	0	0	0	10
Injection of Umbradil 50 % of 38° C with ligation of external carotid artery					
Appl. time 20 sec	0	1	0	0	2
Appl. time 40 sec	0	3	0	0	2

length of the application time at injection into the common carotid artery of Umbradil did not seem virtually to influence the length of the time for the return of blood to the pial vessels. Certainly this time is longer on injection of cooled Umbradil with an application time of 20 seconds but this is mostly due to a rather long time lag in one experiment before the blood returned. At injection with ligation of the external carotid artery, the few experiments made do not indicate that the length of the application time influenced the time for the return of the blood after the end of injection. It seems that at injection of Umbradil with ligation of the external carotid artery the blood returned later than at the corresponding injection of saline. However, the material is too small to admit of any conclusions.

The character of the staining of the dura mater is reviewed in table 19.

DISCUSSION

In accordance with BROMAN & OLSSON (1948) the parameter of the dosage used in this investigation has been the application time. BLOOR, WRENN & MARGOLIS (1951) administered the contrast medium in mg/kg body weight on injection with ligation of the external carotid artery.

In addition to the concentration, the amount of the contrast medium and the time for its action on the brain would be of value for an exact description of the dose of the damaging agent on intracarotid injection in BBB experiments.

The observations of BROMAN & OLSSON (1948, 1949) argue for the length of the application time as one crucial factor for the production of an impairment of the BBB on intracarotid injection. In the present investigation the amount of injected solution was proportional to the length of the application time. BROMAN, FORSSMAN & OLSSON (1950), however, found that a given amount of Umbradil 50 % injected into the common carotid artery with a short application time did not give any lesion. However, it did so after prolongation of the application time by arresting the cerebral circulation, from a non-injurious to an injurious length of the application time. This would imply that the injected amount was of minor importance than the length of the application time for obtaining a lesion. The amounts injected were about half of the volume required for obtaining an application time of 5 seconds in the present investigation.

BLOOR, WRENN & MARGOLIS (1951) found that the same dose of contrast medium in mg/kg body weight less frequently produced a damage to the BBB when the injection was performed under carbon dioxide inhalation. They attributed this effect to the increased blood flow under carbon dioxide administration and regarded their experiment as "the converse experiment" of BROMAN, FORSSMAN & OLSSON (1950). This would mean that the time for the action of the contrast medium, which was not investigated, would have been shorter during carbon dioxide administration.

Thus the concentration and the length of the application time would be 2 decisive factors in producing a lesion. The injected amount of the contrast medium would be of less importance, particularly on injection into the common carotid artery, in which case that part of the injected amount which enters the brain vessels is unknown. The only possible way to measure the dosage in such experiments is to record the application time and the concentration used. In the present experimental series the concentration of the contrast medium was usually 50 per cent. Thus the effect of different concentrations of the contrast medium has not been studied. Such studies have, however, been performed by earlier

investigators such as BROMAN & OLSSON (1948, 1949) and BLOOR, WRENN & MARGOLIS (1951), who showed that with increasing concentration the lesion was generally more pronounced.

The possibility that the injected contrast medium might be diluted has been discussed already by BROMAN & OLSSON (1949), STEINWALL (1958) and MARGOLIS *et al.* (1958).

The degree of dilution is not easy to evaluate. The degree of expelling of the blood from the pial vessels was recorded in the present investigation. Intermittent bloodlessness in the veins or bloodlessness only in the arteries for part of the application time did not influence the degree of injury as compared with experiments in which there was bloodlessness in both arteries and veins during the application time. In only one experiment did intermittent expelling of the blood during the injection occur in the arteries without any perceptible effect on the degree of lesion. However, the expelling of the blood from arteries and veins was complete during 10 seconds, which is enough to provoke a lesion just as pronounced as in the case of an application time of 20 seconds.

BROMAN & OLSSON (1949) pointed out that incomplete expelling of the blood in the brain vessels was an important source of error. They found that the degree of the lesion was dependent of the application time and concentration and only experiments with incomplete expelling of the blood were exceptions to this rule. However, they did not more closely define the meaning of 'incomplete replacement.' To judge from the present investigation it did not seem as if the degree of expelling of the blood had any influence upon the degree of injury, provided the blood in the arteries was expelled. Another situation arises when no expelling of the blood occurs. This will be discussed later on.

STEINWALL (1958) injected contrast media into the common carotid artery with ligation of the external carotid artery and found that the concentration required for provoking a lesion was lower than in comparable experiments by BROMAN & OLSSON (1956 b) with injection into the common carotid artery. He explained this difference by assuming that an uncontrollable dilution is less likely on injection with ligation of the external carotid artery. The same opinion was expressed by MARGOLIS *et al.* (1958).

The question arises as to whether the amount of contrast medium that passes through the cerebral vessels in the two different types of experiments is of the same magnitude. It seems reasonable to assume that with the same injection pressure relatively more contrast medium would pass through the cerebral vessels when the external carotid artery is ligated than when it is not ligated. The length of the time for refilling of the pial vessels with blood was not substantially increased when the

external carotid artery was ligated. This means that an increase in the degree of the lesion on injection with ligation of the external carotid artery cannot be attributed to a longer duration of the effect of the contrast medium on the brain with this mode of injection. An incomplete expelling of the pial vessels could occur also at injection with ligation of the external carotid artery. It could not be ascertained that such incomplete expelling perceptibly influenced the degree of the lesion. It proved to be difficult to obtain the predetermined application time at injection of Umbradil 50 % of body temperature into the common carotid artery. This difficulty however, did not exist when the external carotid artery was ligated. This is in agreement with the observation of STEINWALL (1958) that it was easier to expel all the blood with this latter mode of injection.

BROMAN & OLSSON (1948) noticed that the pial vessels did not refill with blood until 1 to 2 seconds after the completion of the injection of saline. On injection of the contrast medium they found the corresponding time to be 3 to 8 seconds. In the present investigation the time for the return of the blood in the pial vessels was about the same as that just mentioned, independently of whether the external carotid artery was ligated or not. Owing to the fact that few comparable series of saline injections had been performed, it could not be shown whether the time for refilling of the blood was longer at injection of Umbradil than of saline, even though such a tendency could not be excluded.

It occasionally happened that the decided application time was not achieved in spite of an uneventful pilot injection. This was particularly the case in the series with Umbradil 50 % of body temperature, when the injection was made into the common carotid artery in contrast to Umbradil 50 % of room temperature. Owing to ligation of the external carotid artery, the predetermined application time was achieved with Umbradil 50 % of body temperature. The same reasoning as that applied in the case of saline in chapter III can be used here and only the possible difference between the effect of Umbradil 50 % of room and of body temperature on the reactions of the vessels will be discussed. The external carotid artery may be more dilated by Umbradil of body temperature than by Umbradil of room temperature as compared with the internal carotid artery.

No investigation in which particular attention is paid to the effect of the contrast medium on the external carotid artery has yet been reported. Contrast medium of the Umbradil type induces a fall in blood pressure on intravenous injection, presumably due to a peripheral vasodilatation (HEATHCOTE & GARDNER, 1933; EDWARDS & BIGURIA, 1934; WEATHERALL, 1942; PAGE, SOMERS & FIELDING, 1948; COTRIM, 1954 and others).

It is not possible to deduce from the communications of these authors whether the temperature of the contrast medium plays a part in eliciting this effect. WEATHERALL (1942) found a vasodilatation on injection into the femoral artery in the dog. On intra-arterial injection of Triurol and Hypaque into a hind leg in the cat LINDGREN & TÖRNELL (1958) found that a solution of room temperature and a slightly heated one gave the same results.

On intracarotid injections of contrast media of the Umbradil type with concomitant studies of the reaction of the cerebral vessels BLOOR, WRENN & MARGOLIS (1954), SCHMIDT (1955), RUSSEL (1959) and KÅGSTRÖM, LINDGREN & TÖRNELL (1960) found a dilatation of the cerebral vessels. FOLTZ, THOMAS & WARD (1952) however, found an initial contraction of the pial vessels followed by a dilatation in the monkey and the cat. Furthermore, they showed that Diodrast of about 21° C would give a relatively more pronounced initial contraction of the cerebral vessels than Diodrast of body temperature, and the latter a relatively more pronounced dilatation of the cerebral vessels than cold Diodrast. INGVAR & SÖDERBERG (1957) found an initial cerebral vasoconstriction on injection of Umbradil of room temperature, which was explained by SÖDERBERG & WECKMAN (1958) as being due to a redistribution of blood into the external carotid artery owing to dilatation of this vessel.

Thus most of the data in the literature support the opinion that a contrast medium of the Umbradil type induces a dilatation of both peripheral and cerebral vessels. The possibility that Umbradil of room temperature might induce a vasoconstriction of the cerebral vessels cannot, however, be wholly excluded. There is no support for the opinion that Umbradil of body temperature exerts a more pronounced dilatation than does Umbradil of room temperature on the peripheral vessels.

In 3 experiments with injection of Umbradil 70 % of 38° C into the common carotid artery with an application time of 20 seconds it was observed that no difficulty existed in obtaining the decided application time of 20 seconds although the contrast medium was of body temperature (JEPSSON, unpublished data). Thus it was found that the possibility of obtaining the predetermined application time with Umbradil was greater when the viscosity of the solution increased (see chapter I). It would seem that a higher viscosity of the injected solution diminishes the possibilities of the blood to enter the circle of Willis from the contralateral internal carotid artery and the vertebral artery and to wash away and dilute the injected solution.

Only on one occasion was no application time at all achieved on injection into the common carotid artery. The brain was stained in the left olfactory bulb only. The olfactory bulbs are supplied from the external

carotid artery (JEPSSON & OLIN, 1960). For some reason the contrast medium did not pass up into the internal carotid artery. This may occur without any obstacle in the internal carotid artery, as observed by JEPSSON & OLIN (1960).

The rise in the blood pressure during the injection of contrast medium into the common artery was generally about 30 to 40 mm Hg. The height of the rise showed some inconsistencies as in 3 experiments with an application time of 10 seconds the rise was only 10 mm Hg or even less. These animals had a higher blood pressure level before the injection than the others in the same series. However, in the other series no tendency towards a relation between blood pressure level before injection and height of blood pressure rise during the injection was found. It could not be shown that the height of the rise was dependent on the length of the application time. At injection with ligation of the external carotid artery the blood pressure rise was lower than at injection into the common carotid artery. This was not due to the difference in temperature of the injected Umbradil since Umbradil of body temperature also produced a rise in blood pressure during the injection. At injection into the common carotid artery the blood pressure increase was as a rule higher with Umbradil than with saline. On ligation of the external carotid artery there seemed to be no difference between the effect of saline and Umbradil on the blood pressure rise, which was generally lower than at injection into the common carotid artery. It was found that the blood pressure rise was significantly lower in the series treated with hexamethonium bromide than in the controls.

The damage to the BBB occurred independently of whether the blood pressure rise during the injection was elicited or not.

On intracarotid injection of contrast media of the Umbradil type in doses of 1 to 5 ml, a fairly small fall in the blood pressure as a rule occurred (SCHMIDT, 1955; INGVAR & SÖDERBERG 1957; SÖDERBERG & WECKMAN, 1958; KÅGSTRÖM, LINDGREN & TÖRNELL, 1960). The fall in the blood pressure was most pronounced after the injection, even though the fall might start during the injection. On intracarotid injection of Diodrast 35 % of 37° C in doses of 1 to 3 ml in cats, RUSSEL (1959) generally found a rise in the systemic arterial pressure of several mm Hg followed by a decrease of about 10 mm Hg. He attributed the rise in blood pressure to the increase in circulatory blood volume. The authors mentioned did not bleed the animals during the injection. In the present investigation a considerable fall occurred after the injection, but this might be due to the loss of blood.

On intracarotid injection with ligation of the external carotid artery of contrast media of the Umbradil type in the cat, WEATHERALL (1942)

found a rise in the blood pressure followed by a fall. With the just mentioned mode of injection BLOOR, WRENN & MARGOLIS (1954) found that Diodrast, in doses up to 5 ml in rabbits, produced only small blood pressure changes. This is in agreement with the results obtained in the present experiments with this mode of injection.

It seems as the blood pressure response produced by Umbradil changes character when the dose is high and the animals bleed at the same time.

LINDGREN & TÖRNELL (1958) ascribed the effect produced by intracarotid injection of Triurol in the cat to an action on vasomotor structures in the brain. It cannot be excluded from the present investigation that part of the blood pressure increase might be elicited from the brain as it occurred also at injection with ligation of the external carotid artery. As the innervation of the carotid sinus was kept intact, the possibility of an excitatory effect on e.g. the chemoreceptors exists. However, as the blood pressure rise was higher when the external carotid artery was not ligated, it seems as if the blood pressure increase might partly have been elicited in this artery. HOL (1960) kept a constant flow through the femoral artery by means of an extracorporeal pump and injected Hypaque into the tubing running from the pump to the distal part of the femoral artery. A vasodilatation was produced in the femoral artery but the blood pressure recorded in the brachial artery rose about 20 mm Hg. HOL suggested that the rise in the general blood pressure, which is not seen in free circulation and is probably masked by the peripheral vasodilatation, may indicate a reflex mechanism with peripheral receptors. However, according to HEYMANS & NEIL (1958), high doses of irritating chemical agents may induce reflex circulatory reactions if injected into the arterial circulation. They express the opinion that such reactions are most likely due, not to stimulation of specific chemoreceptors, but to the stimulation of non-specific pain receptors.

Whatever the origin of the actual blood pressure rise, the experiments with hexamethonium treated animals show that it is in part elicited via the sympathetic nervous system, as hexamethonium is a ganglion blocking agent (PATON & ZAIMIS, 1951). The blood pressure rise was reduced in hypothermia (see chapter VI).

The lesion was not only located in the injected internal carotid area but often spread to the vertebral area on the same side, the vertebral area on the contralateral side, and the opposite internal carotid area. A staining of non-injected vascular regions was noted by BROMAN & OLSSON (1948), who attributed the phenomenon to a high injection pressure. The investigation by JEPSSON & OLIN (1960) has further corroborated this opinion and showed that by an increasing effective injection pressure, i.e. the difference between blood pressure and injection pressure during the

injection, the injected substance tended to fill more vascular areas in the brain. The effective injection pressure was not measured in the present investigation, but the order of magnitude was about the same in the different series except the one with treatment with hexamethonium bromide. The variation noted in the extent of the lesion might perhaps be due to other factors, such as anatomic variations in the cerebral vessels or variations in the effect of Umbradil on the tonus of the cerebral vessels alone or in combination with minor differences in the effective injection pressure. Turning of the animal's head to the injected side during the injection did not restrict the lesion to the injected internal carotid area. JEPSSON & OLIN (1960) also found that the specific gravity of the injected medium had no influence upon the extent of the cerebral arteriogram.

The other type of location of the lesion (the injected internal carotid area not the most damaged) occurred in 24 out of 52 experiments. Such a location was also noticed by BROMAN & OLSSON (1948, 1949). This type of location can be divided into 2 groups: 1) The injected internal carotid area is injured but another vascular area is more damaged. 2) The injected internal carotid area is faintly stained or negative and another vascular area is pronouncedly injured. Out of the 24 experiments with an unexpected location of the lesion, 10 belonged to the former group and 14 to the latter (see table 13).

The location of the most damaged area in the region of the vertebral arteries seems to be explained by JEPSSON & OLIN (1960), who found retrograde filling of the vertebral arteries. It can be presumed that the contrast medium passes upwards at the end of the injection and that thus the vertebral area is again exposed to the contrast medium. This would mean that the application time is longer in that region. In the experiments in which the non-injected internal carotid area was the most damaged one, the vertebral area was regularly injured. This might mean either that the contrast medium from the vertebral arteries for some reason flowed into the non-injected internal carotid area rather than into the injected one, or that a retrograde filling of the internal carotid artery on the side opposite to the injection took place.

It is more difficult to explain the faint or absent staining of the region of the injected internal carotid artery in group 2. It must be borne in mind that the blood in the pial vessels on that side was expelled. However, even if expelling of the blood is observed, this does not necessarily mean that the contrast medium is actually moving. It cannot be excluded that the faint staining on the injected side is due to the possibility that only a minimal amount of contrast medium flows through the vessels.

In some of the experiments with faint staining on the injected side

the dura mater was faintly stained on this side too. However, it was found that such faint staining of the dura mater could occur with simultaneous pronounced staining of the brain on the same side. Furthermore, it is not likely that the degree of staining of the dura mater on one side should be related to the staining of the brain on the same side, since the dura mater is supplied from the meningeal artery running from the external carotid artery (TANDLER, 1899). It is more likely that the observed difference in staining between the dura mater on the injected side and the side opposite to the injection might be due to impaired collateral circulation as the common carotid artery is ligated on the side of injection. It never occurred that the dura mater was more faintly stained on the side opposite to the injection (table 19).

It should be added that the degree of injury within the other parts of the brain was compatible with the application time observed. It thus seems as if the obtaining of an application time only means that a lesion occurs somewhere in the brain. Ligation of the external carotid artery, treatment with hexamethonium bromide and turning the animal's head to the injected side during the injection did not diminish the frequency of an unexpected location of the lesion. In the first case the effective injection pressure in the actual experiments was about 100 mm Hg, which according to JEPSSON & OLIN (1960) gives a filling of all cerebral vessels and retrograde filling of the vertebral arteries and the internal carotid artery on the opposite side. The factors facilitating an unexpected location of the lesion according to the arguments given above are thus present. The same applies to the series treated with hexamethonium bromide. It has not so far been investigated whether turning of the animal's head to the injected side implies that the retrograde filling of the arteries running to the brain is facilitated.

The macroscopic appearance of the lesions was as a rule a diffuse blue staining of varying intensity. In some experiments stasis occurred either in an entire vascular area or in part of it. The stasis is probably due to an exsudation of plasma. The investigation of BASSETT *et al.* (1953) who found that Diodrast 35 % could produce an injury to the BBB as shown by Evans blue, and of EDSTRÖM (1961), who found that the lesion produced by Triurol could be indicated with 131 I-tagged human serum albumin, show that the injury to the BBB produced by contrast media is such that serum albumin can pass into the brain parenchyma. MARGOLIS *et al.* (1959) found that the blood flow was slowed down after the injection of Urokon 70 %. A progressive sludging developing into stasis was found.

In some instances the staining was patchy or streaky. This might be due to the possibility that the contrast medium remains for a different

length of time in different vessels, which might possibly explain the non-uniform character of the lesion. Such patchy and streaky lesions were prone to occur in such areas as were more likely to have been exposed to the contrast medium for a shorter time, i.e. the regions bordering on negative parts of the brain. In these areas it is also more likely that the contrast medium might have been diluted by blood.

The microscopic picture of the vital staining of the injury to the BBB produced by contrast medium has been summarily described by BROMAN & OLSSON (1948, 1949) for trpb and by BLOOR, WRENN & MARGOLIS (1951) in the case of tetra-sulfonic acid of copper phtalocyanine. In the present investigation the same general picture was obtained. The vessel wall was always stained in damaged areas. Staining of the endothelial nuclei mostly occurred in the vicinity of areas with stasis. The staining of the parenchyma was both diffuse and located in the parenchymal nuclei. Stasis occurred occasionally and more often with the higher doses of the contrast medium. Staining of the vessel wall was sometimes noticed within areas of stasis. This might be due to the time sometimes necessary for the stasis to develop (MARGOLIS *et al.*, 1959). It does not seem unreasonable that the defective rinsing, regularly occurring within more heavily damaged areas in spite of an almost complete rinsing within undamaged areas, might be due to the fact that the flow of blood has been impeded during the action of the trpb.

BROMAN & OLSSON (1948) coined the term "pure injury to the BBB" meaning that only passage of trpb was seen and no edema, hemorrhages, necrosis of the tissues or thrombosis. So far no investigation seems to have been performed to test whether necrosis occurs in the brain after intracarotid injection of contrast medium. As regards the spinal cord, however, MARGOLIS *et al.* (1958) have shown that in long-term experiments histopathologic changes occur, ranging from foci of destruction to a complete transverse necrosis of the spinal cord. In view of this investigation it seems reasonable to question whether a pure injury to the BBB occurs as defined by BROMAN & OLSSON (1948). It is not clear in what way BROMAN & OLSSON (1948) investigated the occurrence of necrosis. If it is correct to assume that staining of the parenchymal nuclei means death of the cell (see chapter II), the present results with an abundance of staining of parenchymal nuclei with higher doses and a relatively less pronounced occurrence of such staining in lower doses, might imply that in lower doses necrosis does not necessarily take place. This statement needs further confirmation with histopathologic studies in long-term experiments.

No comparison of the degree of lesion within different areas of the brain in cases of damage to the BBB produced by contrast medium has

been reported. In the present investigation various areas were compared with regard to the degree of injury on macroscopic examination and with regard to the microscopic character of the lesion. The thalamus, cerebral cortex, cerebellum and the quadrigeminal bodies were among the most damaged regions macroscopically; the white matter, hippocampus, septum pellucidum and the pons and the medulla oblongata were less markedly stained.

On the microscopic comparison, in which attention was paid only to the occurrence of histologic characteristics, it was found that staining of parenchymal nuclei was a rare feature in the white matter both in the cerebellum and in the hemispheres. This might indicate that the stained parenchymal nuclei usually represent nerve-cells.

In the dorsal part of the hippocampus and in the basal ganglia and the thalamus, staining of the parenchymal nuclei also occurred less often than in the rhinal cortex. This difference is specially noteworthy as regards the thalamus, which on macroscopic ranking was one of the most damaged areas. This indicates that degree of staining and staining of the parenchymal nuclei do not necessarily run parallel, which otherwise seems to be the rule. The hypothalamus, however, might be an exception in the other direction since staining of the parenchymal nuclei was a regular feature, but on the macroscopic ranking was placed in the middle of the 10 areas of the brain investigated.

Stasis seemed to be a rare feature in the white matter in the cerebellum, in the pons and the medulla oblongata. In the white matter of the hemispheres, stasis was rare only in the corpus callosum. The dorsal part of the hippocampus showed a low frequency of stasis.

The above-mentioned difference in damage might be due either to differences in vulnerability between different areas, to differences in vascularity or to such anatomic variations of the vessels and dynamics of flow that the contrast medium either passed through the regions in different quantities or remained there for different lengths of time.

Nothing is known about the vulnerability of the BBB in different areas and the same is true of the passage of contrast medium through different regions.

Concerning the vascularity of different areas of the brain in the rabbit, STEEGMAN & DE LA FUENTE (1959) found that the cortex is much richer in capillaries than the white matter, which is also known to be the fact in other animals (CRAIGIE, 1938). COBB (1929) found in the hippocampus of the rabbit that the length of the capillary per mm³ in the lamina pyramidalis, the gyrus dentatus and the stratum radiatum, decreased in the order given.

In the cat WOLFF (1938) found decreasing relative vascularity in the

parietal cortex, lateral geniculate ganglion, putamen, globus pallidus and the white matter.

On the assumption that the vascularity of the different areas in the cat are representative of the rabbit, the quoted data might be taken as a basis for the following discussion on the relationship between degree of injury and frequency of capillaries in the various areas. The high intensity of the staining in the cerebellum and the cortex seems to be consistent with the high frequency of capillaries in these areas. As far as known, the thalamus, the hypothalamus and the hippocampus are not particularly different as regards frequency of capillaries, yet these areas were rated differently, the thalamus being the area with the highest rank. The fairly low ranking of the basal ganglia and the white matter seems to be consistent with the relatively low frequency of capillaries in these regions. The low ranking of the pons and the medulla oblongata cannot be explained by low frequency of capillaries. It might be possible that this area has been exposed to the contrast medium for a shorter time as it is supplied by vessels originating from the caudal part of the basilar artery. More detailed knowledge of the density of the capillary bed in various areas and their vascular supply and the flow of the contrast medium through them would be necessary for a further evaluation of the factors that determine the degree of the injury in various areas.

SUMMARY

Umbradil 50 % of room temperature regularly produced an injury to the BBB with an application time of 20 seconds at injection into the common carotid artery. With decreasing application time the degree and extent of the lesion diminished only slightly and still occurred regularly with an application time of 5 seconds. Independently of the mode of injection, lesions with an unexpected location could occur. With Umbradil 50 % of body temperature it was not possible to expel the blood in the pial vessels for the desired length of time as regularly as with Umbradil 50 % of room temperature at injection into the common carotid artery. After ligation of the external carotid artery Umbradil 50 % of body temperature regularly expelled the blood in the pial vessels for up to 40 seconds.

At the injection of Umbradil into the common carotid artery a rise of the blood pressure generally occurred, which was definitely reduced in animals treated with hexamethonium bromide.

INJURY TO BLOOD-BRAIN BARRIER PRODUCED BY UMBRADIL IN HYPOTHERMIA

The effects of hypothermia on the living animal have lately been reviewed by, among others, VIRTUE (1955), NEIL (1956), KAYSER (1957), SMITH (1958), COOPER (1959) and COOPER & ROSS (1960). Symposia on the subject have been published in *The Physiology of Induced Hypothermia* (1956) and *British Medical Bulletin* (1961). The pertinent Russian literature dealing with hypothermia has been summarized in *The Problem of Acute Hypothermia* (1960).

VIRTUE (1955) defined hypothermia as a state of body temperature which is below normal in a homeothermic individual. In essence similar definitions have been given by LÖFSTRÖM (1959b), COOPER & ROSS (1960) and EDHOLM (1961).

The general effects of hypothermia may be exemplified by the description of its effects on the rabbit.

After cooling rabbits to a body temperature of about 20° C, WALTHER (1862) found decreased heart rate and respiration rate. The production of urine ceased. He noted that the animals were anesthetized, the blood pressure low and the muscles excitable. The animals could be re-animated by warming or artificial respiration; otherwise they died of lung edema.

PFLÜGER (1878) measured the oxygen consumption of rabbits at a body temperature of 22° C and found it to be a third of the values at normal body temperature. HEYMANS (1921) cooled the animals by means of an extracorporeal circuit and found that they died at rectal temperatures of 18—20° C.

ARIEL, BISHOP & WARREN (1943) cooled unanesthetized rabbits in ice water. Below 20° C the muscle reflexes disappeared. The respiration rate and heart rate decreased to about a third of the normal values. ARIEL & WARREN (1943) found that cooled rabbits died at body temperatures of 10—12° C. The basal metabolic rate fell by 57—97 per cent at 20° C of the initial values at normal body temperature.

NIELSEN (1954) cooled rabbits to a body temperature of about 22° C with an extracorporeal blood circuit and initial Nembutal narcosis. The respiration rate decreased to about 10/min., the pulse to about 50/min. and the blood pressure to about 40 mm Hg. The serum potassium showed a significant decrease, which was avoided by cooling with an artificial

kidney. ANDERSEN & NIELSEN (1955) found impaired renal function in rabbits cooled to a body temperature of about 22° C.

After anesthetizing rabbits with Nembutal, BARTLETT (1957) packed them in crushed ice. The body temperature was measured about 7 cm above the anus. The heart rates, respiratory rates, mean arterial blood pressure and metabolic rates were recorded. The rabbits were cooled to about 20° C in about 100 minutes and to about 12° C in 200 minutes. The heart rate fell from about 300/min. to about 50—60/min. at 20° C and decreased to about 10/min. or lower at about 10° C. The respiratory rate fell from about 60/min. initially to about 10/min. at 20° C and decreased further at lower body temperatures. The blood pressure was about 100 mm Hg before the cooling, and about 50 mm Hg at 25° C and about 20 mm Hg at 20° C. The oxygen consumption was about 25 ml/min. at normal body temperature, about 5 ml/min. at 20° C, and reached zero values at 10° C. The fall in oxygen consumption was not linearly related to body temperature. The material was divided into 2 groups with regard to body temperature at death. It was found that the blood pressure, heart rate and respiratory rate were significantly more depressed in the animals that died above a body temperature of 11° C than in animals dying at a lower body temperature. In only one out of 27 rabbits ventricular fibrillation occurred.

In rabbits anesthetized with ether and cooled to a body temperature of about 20° C, LÖFSTRÖM (1957, 1959a, b) found a decrease of the total plasma volume. He described intravascular aggregation at a body temperature of 32 to 26° C, which increased to stagnation of red blood cells at 20° C. The oxygen consumption rate fell linearly to 20° C. During arrested rewarming from hypothermia the rapid disappearance of intravascular aggregation produced by infusion of low molecular dextran was accompanied by an increase of oxygen consumption.

HALL, BJÖRKMAN, JOHANSSON, JUHLIN & TIBBLIN (1960) found no changes ascribable to hypothermia in heart, lungs and spleen in the rabbit cooled to 28° C on macroscopic and microscopic examination.

Several investigations have been carried out in order to study the effects of hypothermia on the central nervous system. SIMPSON & HERRING (1905) and BRITTON (1923) agreed that the reflexes disappeared on cooling and again appeared in a certain order on rewarming.

FAZEKAS & HIMWICH (1939) observed that in dogs subjected to hypothermia down to 26° C, the cerebral arteriovenous oxygen difference was diminished as the rectal temperature fell. The authors concluded that a great diminution of cerebral metabolism took place as a result of the fall of temperature. The decreased blood flow in hypothermia would have acted in the opposite direction increasing the arteriovenous oxygen

difference. Similar results were obtained in the dog by BIGELOW, LINDSAY, HARRISON, GORDON & GREENWOOD (1950), ROSOMOFF & HOLADAY (1954), KLEINERMAN & HOPKINS (1955) and LOUGHEED & KAHN (1955), and in the monkey by BERING, TAREN, McMURREY & BERNHARD (1956).

A reduction of the cerebral blood flow in hypothermia (25°C) to about a third of the normal values in the dog was found by ROSOMOFF & HOLADAY (1954), KLEINERMAN & HOPKINS (1955), and in the monkey by BERING *et al.* (1956).

A doubling of the cerebral vascular resistance in hypothermia of about 25°C in the dog was observed by KLEINERMAN & HOPKINS (1955), ROSOMOFF (1956a) and in the monkey by BERING *et al.* (1956). Similar results were obtained by STERN & GOOD (1960) in the monkey and the cat. On selective cooling of the brain SENNING & OLSSON (1956), LUND, JOHANSEN, KROG & BIRKELAND (1958) and LOURIE, HOLMES, WEINSTEIN, SCHWARTZ & O'LEARY (1960) found a decrease of the vascular resistance with falling temperature as measured by the carotid perfusion pressure. In hypothermia (25°C) in dogs, ROSOMOFF & GILBERT (1955) found that the cerebrospinal fluid pressure fell to about half of its initial value. There was also a decrease in brain volume.

KLEINERMAN (1956) demonstrated that during hypothermia (25°C) in dogs and monkeys, a rise in alveolar CO_2 -tension causes a vasodilatation in the brain. In studies of cats and monkeys of body temperatures down to 25°C , MEYER & HUNTER (1957) found a reduction in the caliber of the cortical arteries by 50 per cent. The intracranial pressure in hypothermia was slightly reduced. GURDJIAN, THOMAS & WEBSTER (1960) cooled monkeys to body temperatures of 10 to 2.8°C . The caliber of the pial vessels was not reduced below temperatures of 30°C . Not even at temperatures of 2.8°C was any major change in caliber recorded.

A number of investigations have shown that the electrical activity of the brain diminishes in frequency and amplitude with decreased body temperature and finally disappears at about 20°C (LIBET, FAZEKAS, MEIROWSKY, CAMPBELL & HIMWICH, 1940; TEN CATE, HORSTEN & KOOPMAN, 1949; CALLAGHAN, McQUEEN, SCOTT & BIGELOW, 1954; GURDJIAN, THOMAS & WEBSTER, 1960 and others). In rabbits cooled without narcosis to body temperatures below 30°C , NOELL, BRILLER & BRENDEN (1952) found that the threshold for inducing seizures with Metrazol was markedly lowered. On electrical stimulation in the temporal lobe, however, BALDWIN, FROST, WOOD & LEWIS (1956) could not provoke epileptiform activity in the chimpanzee in hypothermia (25°C). In monkeys, CALLAGHAN *et al.* (1954) found that lowering of the body temperature to 20°C had no effect on their ability to perform tests learned prior to the experiment. ANDJUS, KNÖPELMACHER, RUSSEL &

SMITH (1955) found a tendency to impaired memory in rats after cooling to a body temperature below 15° C.

No deleterious effects on the brain produced by hypothermia (20° C) seems to be reported. However, on cooling brains of dogs selectively by extracorporeal circulation to temperatures below 12° C, PARKINS, JENSEN & VARS (1954) found that after rewarming, the animals showed evidence of gross neurological damage such as severe motor ataxia or marked confusion. WOODHALL, REYNOLDS, MAHALEY & SANDERS (1958) and LOURIE *et al.* (1960) did not report any unfavourable effects of selective brain cooling in dogs to temperatures of about 20° C provided the perfusion flow was kept low (WOODHALL *et al.*, 1958). NIAZI & LEWIS (1956) induced hypothermia in dogs (body temperature below 10° C) by surface cooling. In 3 out of the surviving 16 animals, neurological damage was found, which they considered due to the rewarming procedure. Dogs cooled to a body temperature of about 5° C with extracorporeal circulation showed no clinical evidence of cerebral damage (KENYON & LUDBROOK, 1957; KENYON, 1961).

The experience gained by experimental investigations of animals indicates that during hypothermia the brain is less vulnerable, particularly to anoxia. This was first shown by BIGELOW, CALLAGHAN & HOPPS (1950), who found that dogs cooled to 20° C could survive a 15-minute period of occlusion of the circulation. On selective cooling of the brain in dogs, PARKINS, JENSEN & VARS (1954) found that brain temperatures of about 20° C and rectal temperatures of about 32° C seemed sufficient to protect the animal against 39 minutes of complete circulatory occlusion. GOLLAN (1954) reported survival of dogs cooled by extracorporeal circulation to heart temperatures of about 0° C and submitted to a cardiac standstill of about one hour. No clinical signs of damage were observed. LOUGHEED & KAHN (1955) found that dogs survived 15 minutes of anoxia produced by breathing 100 per cent nitrogen at body temperatures of 25° C.

ROSOMOFF (1956b) studied the effect of ligation of the middle cerebral artery in dogs at an oesophageal temperature of 22—24° C. In normothermic animals a cerebral infarction regularly developed, while in hypothermic animals there was either none at all or only a small sized lesion.

McMURREY, BERNHARD, TAREN & BERING (1956) found that the afferent circulation to the brain of monkeys could be occluded for 15 minutes but not for 20 minutes at body temperatures of about 22—24° C. Multiple periods of afferent cerebral vascular occlusion during 15 minutes resulted as a rule in obvious cerebral damage as judged from EEG and clinical appearance. If the duration of each multiple period was reduced

to 12 minutes the occlusion was tolerated if an adequate time for recovery was allowed between periods of occlusion. Similar results were obtained by PORTER & HAYES (1959).

ROSOMOFF (1957) produced an acute cerebrovascular occlusion in dogs by resecting the left middle cerebral artery from its origin to the first major cortical bifurcation. Hypothermia was then induced after delays from 0 to 20 minutes following the initial occlusion of the artery. The animal was kept at a body temperature of 24°C or less for an hour, and the dog was then rewarmed to normothermic levels. It was demonstrated that protection against infarction was obtained if the induction of hypothermia was begun within 15 minutes after the onset of the occlusion and if a temperature of 24°C was reached with a total lapse of time of 90 minutes.

PONTIUS & DEBAKEY (1956) showed that dogs in hypothermia of about 25°C could tolerate an occlusion of the blood supply to the spinal cord or the brain for 60 and 30 minutes, respectively, which at normal body temperature was incompatible with life or gave severe neurological disorders. SPOHN, KOLB, HEINZEL, KRATZERT & WENZ (1959) found that dogs cooled to a rectal temperature of about 19°C could tolerate 60 minutes of occlusion of the circulation, but that longer occlusions caused convulsions and death on rewarming. NIAZI & LEWIS (1957) found no sign of brain damage in monkeys after surface cooling to a body temperature of $11\text{--}14^{\circ}\text{C}$ and a cardiac standstill from 1/2 to 2 hours.

RAIMONDI, CLASEN, BEATTIE & TAYLOR (1959) made local cooling of the brain in rabbits and found that the lesion was less pronounced if the damage was inflicted during hypothermia. Mortality was lower in animals that had got prednisolon the day before the hypothermia than in the group just mentioned. ROSOMOFF (1959) performed similar experiments, inflicting standardized brain injuries in normothermic and hypothermic dogs by application of liquid nitrogen in a brass cylinder to the exposed dura mater and the subjacent brain. At normal body temperatures the lesion was hemorrhagic with a post-traumatic inflammatory reaction. When brain injuries of the same size were produced at a body temperature of 25°C or less, the lesion was only partially hemorrhagic and the post-traumatic cerebral edema was markedly reduced. Using the same technique ROSOMOFF, SHULMAN, RAYNOR & GRAINGER (1960) studied the median lethal injury in normothermic dogs. In dogs of a body temperature of 25°C or less lesions of this kind were produced and the animals were kept at this low temperature for 1 hour, after which they were rewarmed and observed for 7 days. Out of 15 dogs, 14 survived. Hypothermia was induced 1 to 12 hours after

the injury and the temperature maintained at 25° C for 1 hour. It was found that all animals survived in which the induction of hypothermia had begun within 3 hours after the injury. The first death occurred when the induction of hypothermia was delayed 4 hours.

MALMEJAC, NEVERRE, PLANE, MALMEJAC & FREDENUCCI (1959) cooled dogs and monkeys to body temperatures of 18—20° C and clamped the arterial cerebral circulation for 30 minutes without deleterious effects. Cooling to a body temperature of 12° C caused delayed recovery extending over 4 days after rewarming.

Investigations of the BBB in hypothermia have been few. In preliminary studies JEPSSON & NIELSEN (1955) described the effect of a contrast medium on the BBB in hypothermia. In these experiments the cooling was performed by means of circulating cold air which gave a cooling time to 25° C of about 3 hours. LASKOWSKI (1959) and LASKOWSKI, KLATZO & BALDWIN (1960) induced cerebral edema in the cat by freezing the cortex, after which the BBB was studied with sodium fluorescein. The body temperature was lowered by surface cooling. If the lesion was inflicted during hypothermia (26° C), which was maintained for 6 hours, the lesion was delayed for 24 hours as compared with the normothermic animals. It was also less pronounced than in the control group. Animals damaged by freezing at a normal body temperature and then cooled 6 hours later to body temperatures of 25—26° C and kept at that temperature for 48 hours, showed a degree of edema resembling that after six hours in normothermic animals.

LOURIE, WEINSTEIN & O'LEARY (1960) cooled cats, anesthetized with Nembutal, in water to brain temperatures from about 20° C to about 3° C. Trpb was administered either by repeated injections over a period of days or in single doses at the experiments. Rinsing and fixation were done via the aorta. Microscopic studies of the brains were performed. Methylene blue was used in experiments on the vital staining and was found to exert the same effect in normothermic and hypothermic animals down to a temperature of 12.5° C. Cats with brain temperatures of 17—19° C received 4 ml of a 1 per cent trpb solution intravenously during the period of hypothermia. Most of the animals had received 4 ml of the trpb solution intraperitoneally 3—4 days prior to cooling. They were killed 6 to 72 hours after hypothermia. During this period the dose of trpb was repeated in intraperitoneal injection in the animals living 24 hours and more. The authors reported that none of the brains showed any blue discoloration except in the hypophysis, choroid plexuses, etc. Staining was produced in the brain of cats in which injury to the BBB was produced by concentrated angiographic dyes at brain temperatures of 20° C. Six new-born pups were not found to be stained by trpb when

hot needles were inserted at brain temperatures of 13—16° C. Supravital staining of cats at brain temperatures of 2.5—8° C was of a very low degree after perfusion with 40 ml 0.15 per cent trpb solution via the aorta. The results were confirmed by vital staining at brain temperatures of 9° C, when 4 ml, trpb solution, 1 per cent was given intravenously. The circulation was maintained for at least 10 minutes following injection of the trpb solution. Selective brain cooling in dogs showed no damage to the BBB when 100 ml of a 0.15 per cent trpb solution was administered into the carotid artery at a brain temperature of 13° C. On survival from 18 to 76 days no passage of trpb into the brain was found when given in doses of 30—50 ml in 1 per cent solution 30 minutes before sacrifice.

KENAN *et al.* (1958) injected Urokon 70 % into the aorta in the dog. The degree of the lesion in the spinal cord was studied by histopathologic methods. The spinal cord was removed 4—6 days after the injection and any lesion studied by histologic methods. Hypothermia (27—29° C) during the injection of Urokon and one hour after, decreased the degree of the lesion. The expelling of the blood in the spinal vessels was not checked and the authors did not study the possible effect of the changes in the blood in hypothermia on the passage of the contrast medium through the vascular bed.

The effect of extracorporeal cooling on the BBB as studied with sodium fluorescein was investigated by POLLARD *et al.* (1961) in the dog at oesophageal temperatures of 12° C. The impairment of the BBB varied with the technique of extracorporeal circulation used. The warming procedure seemed to increase the lesion. Owing to the actual experimental conditions an impairment of the BBB was taken as presumptive evidence of gas embolism.

The purpose of the experiments referred to in the present chapter was to study the effect of Umbradil 50 % on the BBB in hypothermia (25° C) obtained by surface cooling in water, the application time being 20 seconds. As will be shown, the damage was less than that in normothermia. Yet it differed from the results obtained in the preliminary experiments in which the damage was nil as seen on macroscopic examination. In order to ascertain whether this discrepancy was due to the difference in length of cooling time, this was prolonged, firstly by cooling the animals in the ordinary way and delaying the time from the end of the cooling to the injection by keeping the animals at a body temperature of 25° C until about 3 hours had elapsed from the start of the cooling, secondly by prolonging the cooling time by using water of a higher temperature in order to obtain a length of time of about 3 hours from start of cooling to the injection. As these procedures did not further

diminish the degree of injury, the experiments were performed at a body temperature of 20° C. At this body temperature the damage produced by Umbradil 50 % was found to be very slight.

In order to examine the relationship between degree of injury and length of application time, experimental series with application times of 15 and 40 seconds were performed. The influence of the temperature of the Umbradil on the lesion was studied by injection of Umbradil 50 % of 38° C with an application time of 20 seconds. In order to test whether it was possible to obtain the same degree of lesion in hypothermic as in normothermic animals, the lesion produced by Umbradil 70 % of 38° C was investigated. The effect of the ligation of the external carotid artery was also investigated. The possible effect of the intravascular aggregation on the lesion was studied by treating animals with low molecular dextran during the cooling. The importance of the length of action of the trpb for the degree of injury was examined. The effect of the blood pressure at the injection on the degree of the lesion was studied by treating animals with noradrenaline. The importance of the blood pressure during the action of trpb was studied by comparing experiments from the dextran treated series with those performed at normal body temperature with injection of hexamethonium bromide.

A. BODY TEMPERATURE 25° C

I. INJECTION OF UMBRADIL 50 % INTO COMMON CAROTID ARTERY

Ordinary cooling

Experimental. — Thirteen rabbits were cooled to about 25° C. Umbradil 50 % of room temperature was injected into the left common carotid artery. The application time was 20 seconds. The blood pressure was not measured in 3 experiments. The brain temperature was recorded in 4 animals before the injection of Umbradil. The animals were killed about 30 minutes after the beginning of the infusion of trpb. The brain was cut, 6 frontal sections being made through the cerebrum. If damaged, the cerebellum was sectioned. No colour photographs were taken.

Results

The experimental data are given in tables 66 and 67. The cooling in water was finished when the animals had reached a body temperature with a mean of about 26° C, which on an average occurred about 40 minutes after the beginning of cooling. The brain temperature was found to be the same as the rectal temperature in 3 experiments. In the 4th

the brain temperature was 0.7°C lower. At the injection the rectal temperature was on an average 24.8°C and the time from the beginning of the cooling had a mean of about 80 minutes. The body temperature fell by $0.5\text{--}1.0^{\circ}\text{C}$ towards the end of the experiment. The blood pressure at the beginning of the injection ranged from 15 to 85 mm Hg. One animal (C 26) got convulsions 5 minutes after the injection of Umbradil. The blood pressure was generally 10—20 mm Hg lower after the injection and fell towards the end of the experiment to values ranging from 10 to 45 mm Hg. The trpb infusion was on an average completed in about 14 minutes. All animals but 2 were killed 30 minutes after the beginning of the trpb infusion. All animals but 3 had audible heart-beats immediately before they were killed.

The determined application time of 20 seconds was achieved. In 3 animals the blood was expelled from the arteries only.

Macroscopic examination. — The choroid plexuses, the median eminence and the area postrema were stained blue. The degree of the injury is given in table 67. The staining was as a rule patchy or streaky and confluent only in C 28. The cortex was the most regularly stained area. The track of the needle for the temperature recording was stained blue.

In 4 experiments the left internal carotid area was the most heavily damaged. In 3 experiments (C 23, C 27, C 37) the right internal carotid area was more damaged than the left internal carotid area and in C 26 the left vertebral area was more stained. With the exception of C 27 the differences between the mentioned areas were small.

Microscopic examination. — In the experiment with a pronounced lesion (C 28) the vessel walls were stained and definite staining of the parenchymal nuclei was found. In the experiments with a low-grade lesion, the vessel wall was stained and the neighbouring parenchymal area was stained a diffuse blue. Occasional staining of the parenchymal nuclei occurred. Such staining was almost only seen in the cortex. As a rule, staining of the vessel wall without passage of trpb into the parenchyma was seen but seldom. In the negative areas the vessel wall was generally unstained. In experiments in which staining of the vessel wall occurred in negative areas, the staining was mostly confined to the larger parenchymal vessels. In C 23 the ependyma of the lateral ventricles was slightly and diffusely stained. Staining of the vessel wall of the pericallosal artery could occur, but this was not the rule.

Prolonged cooling — injection time

1. Prolonged cooling by delay between end of cooling and injection

Experimental. — Five rabbits were cooled in water of about 10°C . When the cooling in water was finished at $25\text{--}26^{\circ}\text{C}$ the animal was

kept at 24—25° C for about 2 hours until the BBB experiment was performed. The body temperature was kept at that level by heating the operating table when the temperature began to fall. In case a tendency to rise was observed, the animal was covered with a towel soaked in cold water.

Umbradil 50 % of room temperature was injected into the left common carotid artery with an application time of 20 seconds. The animals were intended to live 30 minutes after the injection. The brain was cut, 2—3 sections being made from 3 parts of the brain (prosencephalon, quadrigeminal bodies, cerebellum).

Results

The experimental data are given in tables 68 and 69 and are in agreement with those obtained in the previous series with cooling to 25° C. The lowest recorded body temperature between the end of cooling in the water and the injection was 23.7° C. The temperature was not higher than 25° C. The time between start of cooling and injection of Umbradil ranged from 175 to 193 minutes.

The intended application time was achieved. The body temperature at the injection was on an average 24.8° C and fell 0.5—1.0° C towards the end of the experiment. Three animals lived 30 minutes after the injection of Umbradil. One died about 25 minutes and one 6 minutes after the injection. The blood pressure was as a rule low, the highest recorded blood pressure before the injection being about 40 mm Hg.

Macroscopic examination. — The choroid plexuses, median eminence and the area postrema were stained. The results of the rating of the degree of injury on colour photographs and sections are presented in table 69. The tendency to rate the lesion higher on the colour photographs was in part due to the fact that the surface of the brain was more pronouncedly stained than the cut surface. This was most obvious in C 52. Tearing off the pia mater resulted in less pronounced staining of the surface of the brain. In the more intensely stained brains the lesion on the surface was as a rule confluent, whereas in the faintly stained brains the lesion was fairly dotted. On the cut surface the lesion was as a rule streaky, particularly in the cortex, or dotted. The cortex was mostly one of the damaged areas. Staining of the white matter was a rather uncommon feature. In C 54 the left vertebral area was somewhat more intensely stained than the left internal carotid area. In the other experiments no difference between the degree of lesion of the various vascular areas was discernible.

Microscopic examination. — The wall of the larger parenchymal vessels was often stained in the endothelial nuclei, but sometimes only a diffuse

staining of the vessel wall occurred. The vessels were well rinsed. Staining of the parenchyma was rather an exception and was faint also on investigation in reflected light. This was also true of C 52, which showed the most pronounced staining on the cut surface. The superior olivary nucleus was stained in the parenchymal nuclei in C 53. In this brain it was also possible to see with certainty that the pia mater, but not the parenchyma, was stained blue. The blue area in C 51 consisted of a vessel filled with red blood corpuscles, and the nuclei of the immediate surrounding parenchyma were stained.

2. Slow cooling

Experimental. — Six rabbits were cooled in water at a temperature of about 21—25° C in order to prolong the cooling time. At a body temperature of 26—27° C the cooling in water was finished and the cooling pursued on the operating table for another 30—45 minutes when the preparation was started. Thus a length of time of 2.5—3 hours from the beginning of cooling to the injection was obtained. Umbradil 50 % of room temperature was then injected into the left common carotid artery with an application time of 20 seconds. The intention was to kill the animals 30 minutes after the beginning of the trpb infusion. The brain was cut, 2—3 sections being made from 3 different parts of the brain (prosencephalon, quadrigeminal bodies and cerebellum).

Results

The experimental data are given in tables 70 and 71. The cooling time in water had a mean of 103 minutes. The time from the beginning of the cooling to the injection of Umbradil varied from 156 to 210 minutes. The body temperature had a mean of 24.5° C at the injection of Umbradil. The injected amount ranged from 25 to 40 ml and the injection time from 23 to 25 seconds. The decided application time was achieved. No convulsions occurred.

The trpb infusion was finished in 4—18 minutes. The animals were killed about 30 minutes after the beginning of the trpb infusion, except one animal that died 8 minutes after the beginning of the trpb infusion, which was completed in 4 minutes. In C 47 the brain temperature was measured in the right side of the cerebrum after the end of the trpb infusion and was 0.5° C lower than the rectal temperature.

The blood pressure before the injection was about 50 mm Hg with one exceptional value of 90 mm Hg. After the injection it was 20—40 mm Hg lower. It decreased further towards the end of the experiment.

The fall in body temperature towards the end of the experiment was 0.5°C at most.

Macroscopic examination. — The choroid plexuses, the median eminence and the area postrema were stained blue. The degree of the injury as judged from colour photographs and sections is presented in table 71. On the surface the staining was as a rule confluent but also patchy and dotted, particularly in areas less intensely stained. C 47 had a red appearance, similar to that in stasis, on the convexity of both hemispheres. On the cut surface the lesion often consisted of streaky or dotted areas, sometimes also of smaller confluent stained areas. In C 44 the staining within the left cortex was very intense and confluent. The cortex was as a rule one of the stained areas. In C 48 a small area in the cortex beneath the trephination was stained a faint blue.

The difference in degree of lesion between the various areas of vascular supply was generally small. In C 47 the left vertebral area appeared more stained than the left internal carotid area.

Microscopic examination. — In general, in the macroscopically damaged areas, the vessel wall was diffusely stained and surrounded by a diffuse blue staining of the parenchyma. The parenchymal nuclei were often stained. The vessel wall was mostly stained only in damaged areas. The rinsing of the vessels was good except in the case of C 47.

In C 44 the left cortex had an appearance not distinguishable from a damage to the BBB at normal body temperature. No microscopic sign of stasis was seen in C 47. The stained area in the cortex of C 48 consisted of stained vessels and blue parenchymal nuclei in the immediate vicinity of the vessels. The wall of the pericallosal arteries was as a rule diffusely stained. Staining of the nuclei of the endothelium in the intracerebral vessels was a rare feature in this group.

B. BODY TEMPERATURE 20°C

I. INJECTION INTO COMMON CAROTID ARTERY

Umbradil 70 % of 38°C

Experimental. — Five rabbits were cooled and prepared. The Umbradil ampoules were heated to 38.5°C and the injection into the left common carotid artery was made with an application time of 20 seconds. The intention was to kill the animals 30 minutes after the beginning of the trpb infusion. The brain was cut, 3 sections being made from 4 parts of the brain.

Results

The experimental data are given in tables 72 and 73. The cooling time had a mean of 101 minutes. The body temperature at the end of

the cooling in water was about 21°C and was the same at the injection of Umbradil, though it rose about 0.5°C towards the end of the experiment.

The amounts of Umbradil injected ranged from 16 to 28 ml, and the injection time from 22 to 23 seconds. The decided application time was achieved. The blood pressure was as a rule low (about 30 mm Hg) and remained at this level until after the injection. Thus no fall in the blood pressure occurred on removing the jugular clamps. This was the rule in hypothermia. Only in one experiment a pronounced rise (about 30 mm Hg) occurred during the injection of Umbradil. Otherwise this rise was small or absent. The blood pressure during and after the infusion of trpb as a rule ranged from about 20 to 30 mm Hg, also in the animal with a high blood pressure before injection. In C 118 a rise in blood pressure occurred after the end of the trpb infusion. The trpb solution was infused in about 7 minutes and the animals were killed 30 minutes after the beginning of the trpb infusion. One animal died about 17 minutes after the beginning of the trpb infusion.

Twenty minutes after the injection of Umbradil, one animal (C 121) got convulsions which continued for the remaining 10 minutes of the experiment.

Macroscopic examination. — The areas normally permeable to trpb were stained. In C 118 and C 122 the dura mater was not stained, or only faintly so, on the left side. In C 123 the dura mater was patchy blue bilaterally.

The degree of the injury as judged from colour photographs and sections is presented in table 73. The difference in degree of staining between the surface and the cut surface was impressive. Stripping off the pia mater considerably diminished the staining of the surface. On the cut surface the staining was usually only streaky or dotty with the exception of C 118, in which an outer brim of the cortex and the basal ganglia on the left side were stained a diffuse blue (see plate II).

In one experiment, the staining of the various areas of vascular supply was more marked in the region of the internal carotid artery on the side of injection. In the other experiments the difference between the various areas was small, but no area was more stained than the area of the internal carotid artery on the injected side.

Microscopic examination. — The most characteristic feature of the microscopic picture in this group was that pronounced staining of the vessel wall with faint or absent staining of the parenchyma was regularly found, even though parenchymal staining was found in some parts of the brain. The vessel wall was as a rule stained also in the endothelial nuclei on the capillary level (plate III:2). Most of the vessels were stained.

A faint blue staining around the vessels was sometimes seen. Staining of the parenchymal nuclei could also occur in the vicinity of the vessels.

The pia mater generally appeared as a more intensely stained thin brim around the faintly stained parenchyma.

In C 118 a pronounced staining occurred, particularly in the cortex on both sides and in the basal ganglia on the left side. In the right cortex there was even stasis in the parasagittal region in the fore part of the brain and in the cortex of the occipital lobe. The staining of the cortex was most pronounced in the outer layers and appeared as a halo around the vessels.

In C 123 the pronounced staining of the vessel wall without any staining of the parenchyma was extensive. In C 115 the staining of the vessel wall was of the diffuse type, mainly confined to the larger vessels. Many vessels were not stained at all. In this experiment no extravasal trpb was found.

The choroid plexuses were stained diffusely in the epithelium. In one exceptional experiment the epithelial nuclei were stained. The wall of the pericallosal arteries was stained, as were also mostly the endothelial nuclei. The rinsing of the vessels was good with the exception of the most damaged brain, C 118.

Umbradil 50 % of room temperature

1. Application time 20 seconds

Experimental. — Eight rabbits were cooled and prepared. The Umbradil solution was of room temperature and injected with an application time of 20 seconds into the left common carotid artery. The intention was to kill the animals 30 minutes after the beginning of the trpb infusion. The brain was cut, 2—3 sections being made from 3 parts of the brain (prosencephalon, quadrigeminal bodies, cerebellum).

Results

The experimental data are given in tables 74 and 75. The cooling time in water had a mean of 75 minutes. The rectal temperature at the end of cooling was on an average 21.9° C.

At the injection the mean body temperature was 20.5° C and then rose about 0.5° C towards the end of the experiment.

The decided application time was achieved. The amounts of injected Umbradil ranged from 30 to 38 ml. The injection time was about 24 seconds. The blood pressure was very low just before the injection of Umbradil (range 10—30 mm Hg). The blood pressure was low even after

the end of the trpb infusion and rose in one animal only. In the 4 animals that were alive at the end of the experiment the blood pressure was low. The trpb solution was infused in about 10 minutes and the rinsing was started about 30 minutes after the beginning of the trpb infusion in 7 animals. C 62 was rinsed after a trpb duration of 10 minutes. Convulsions did not occur in any animal.

Macroscopic examination. — The choroid plexuses, the median eminence and the area postrema were stained blue. The dura mater was blue in C 64, faintly blue on the right side though even more faint on the left in C 114 and patchy blue in C 61, C 62 and C 59, in the latter mostly on the right side. In the others the staining of the dura mater was not recorded. In C 64 the cortex was stained beneath the burr hole.

The degree of the staining as judged from colour photographs and sections is presented in table 75. The difference in degree between the surface and the cut surface is negligible. The lesion was always faint. The staining was mostly characterized by streaks in the cortex and dots in the basal ganglia, the thalamus and the cerebellum.

The difference in degree of injury between the various areas was almost negligible. In C 114 the staining appeared to be more pronounced within the vertebral area.

Microscopic examination. — The microscopic picture in all the experiments was mainly the same. The walls of the vessels, particularly the larger ones, were stained, either diffusely or in the nuclei of the endothelial cells. The rinsing was generally complete but occasionally trpb and blood corpuscles remained in some vessels. In 4 brains trpb was found in the parenchyma. In one of them the extravasal staining was limited to a few parenchymal nuclei in the quadrigeminal bodies. This damage could not be noted on the macroscopic examination. The staining was faint and confined to the immediate vicinity of the vessels, particularly the larger ones. Occasionally also a few parenchymal nuclei were stained. The choroid plexuses were stained in the epithelium, often with staining of the nuclei. In C 57 the wall of the pericallosal artery was stained.

2. Application time 15 seconds

Experimental. — Five rabbits were cooled and prepared. The Umbradil solution was of room temperature and was injected into the left common carotid artery with an application time of 15 seconds. The intention was to kill the animals 30 or 60 minutes after the injection of Umbradil. The brain was cut, 3 sections being made from 4 parts of the brain.

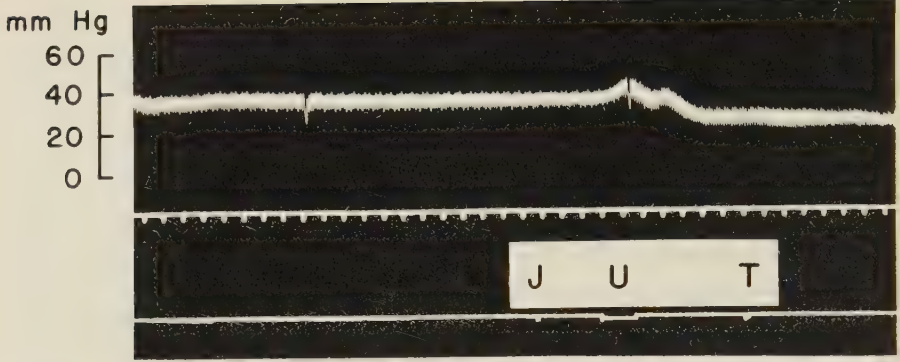


Fig. 4. Hypothermia (20°C). Exp. C 202. Injection of Umbradil 50 % of room temperature into common carotid artery with application time 15 sec. Umbradil 28 ml. Inj. time 16 sec. J: Removal of jugular clamps. U: Injection of Umbradil. T: Start of infusion of trpb. The jugular clamps are replaced between U and T. Time marking: 10 sec. Note the very small blood pressure rise during injection.

Results

The experimental data are given in tables 76 and 77. The mean cooling time was 40 minutes. The rectal temperature at the end of cooling was about 21°C .

At the injection the mean body temperature was 20.4°C and rose about 0.5°C towards the end of the experiment.

The decided application time was achieved. The amount of injected Umbradil ranged from 27 to 35 ml and the injection time from 16 to 22 seconds. The time for refilling the pial vessels with blood after the end of the injection was mostly about 4 seconds. In one exceptional experiment it was 31 seconds (the blood pressure fell to about 10 mm Hg immediately at the end of the injection).

The blood pressure before the injection was about 40 mm Hg. The rise in blood pressure during the injection was small, the highest value being 8 mm Hg (fig. 4). During or after the trpb infusion the blood pressure generally fell below the pre-injection level.

The trpb solution was on an average infused in about 13 minutes. One animal died after the end of the trpb infusion. The others were killed between 30 and 60 minutes after the beginning of the trpb infusion. No convulsions occurred.

Macroscopic examination. — The areas of the brain normally permeable to trpb were stained blue. In C 200 and C 202 the dura mater was faintly stained bilaterally, in C 198 it was faintly stained on the right side and more intensely stained on the left side. In C 204 the staining of the dura

mater was of normal degree on both sides. As seen from table 77 the brains were judged as negative on both colour photographs and on sections.

In C 198 and C 200 slightly blue staining occurred on the surface of the cortex beneath the trephination. In C 204, two faint dots were seen in the fronto-parietal region on the left side of the surface. A few faint dots appeared in the left hippocampus and the left thalamus.

Microscopic examination. — No extravascular staining was seen. In 4 of the brains staining of the walls of the intracerebral vessels occurred on a few occasions in each brain. The staining of the vessel walls was diffuse and only on rare exceptions were the endothelial nuclei stained. The vessels were well rinsed. The wall of the pericallosal arteries was not stained in any of the experiments. The choroid plexuses were stained a diffuse blue in the epithelium.

Umbradil 50 % of 38° C

Experimental. — In order to evaluate the significance of a warm Umbradil solution for the production of the injury to the BBB in hypothermia, 5 rabbits were cooled to a body temperature of about 20° C and Umbradil 50 % heated to about 38° C was injected into the left common carotid artery. The intention was to kill the animals 30 to 60 minutes after the beginning of the trpb infusion. The brain was cut, 3 sections being made from 4 parts of the brain.

Results

The experimental data are given in tables 78 and 79. The cooling time in water ranged from 43 to 63 minutes. The rectal temperature at the end of the cooling was 21° C. The body temperature at the injection of the Umbradil solution was on an average 20.2° C and rose about 0.5° C towards the end of the experiment. The decided application time of 20 seconds was achieved. After the end of the injection of the Umbradil solution it took about 5 seconds before the blood returned to the pial vessels.

The amount of injected Umbradil ranged from 30 to 40 ml. The injection time was on an average about 23 seconds. Before the injection the blood pressure was about 30 mm Hg (range 15—50 mm Hg). The increase in blood pressure during the injection of Umbradil solution ranged from 10 to 16 mm Hg. In 2 experiments the blood pressure fell during the infusion of trpb. In the others the pre-injection level was mostly the same or even higher for the remaining part of the experiment.

The trpb solution was infused in about 12 minutes. One animal died 20

minutes after the beginning of the trpb infusion. In the other 4 experiments the animals lived between 30 and 60 minutes after the beginning of the trpb infusion.

Macroscopic examination. — The areas of the brain normally permeable to trpb were stained blue. The staining of the dura mater was of a normal degree with the exception of C 210, in which the dura mater was faintly blue. The staining was more pronounced on the right side in C 208 and C 210. The result of the assessment of the degree of the lesion is given in table 79. On the surface of C 206, C 208 and C 210 faint staining in areas smaller than one olfactory bulb occurred. On the cut surface, the staining was confined to the pia mater or the outermost layer of the cortex. In the damaged brains the staining was faint and consisted of dots or streaks. In the 3 damaged brains the staining occurred within the left side, but in C 208 and C 212 staining was seen also in the right hippocampus, the right thalamus, and the right hypothalamus, respectively. The staining, however, was not more pronounced on the right side than on the left side.

Microscopic examination. — On microscopic examination, the macroscopically stained areas were seen to consist of the walls of the larger vessels, well rinsed and surrounded by a small, faintly and diffusely stained area. Stained and well rinsed intracerebral vessels were also found within other areas without surrounding parenchymal staining. The walls of the pericallosal arteries were not stained. Remnants of trpb in cortical capillaries were occasionally seen.

The epithelium of the choroid plexuses was diffusely stained. In C 210 staining of the epithelial nuclei was occasionally seen.

Umbradil 50 % of room temperature. Animals treated with low molecular dextran during cooling

As shown by BIGELOW, LINDSAY & GREENWOOD (1950), GELIN & LÖFSTRÖM (1954,1955) and ADOLPH (1956) intravascular aggregation of red blood corpuscles occurs during hypothermia down to about 20° C. At the lowest temperature investigated the intravascular aggregation was of such a degree that the flow of blood through the capillaries was obstructed.

Infusion of low molecular dextran during cooling diminished or abolished the intravascular aggregation during hypothermia (GELIN & LÖFSTRÖM, 1954, 1955; LÖFSTRÖM & ZEDERFELDT, 1957b; LÖFSTRÖM, 1959b).

The biological effect of the intravascular aggregation in hypothermia (about 20° C) on wound healing has been demonstrated in rabbits

by LÖFSTRÖM & ZEDERFELDT (1957a, b, c). They found that the tensile strength of healing wounds, which had been inflicted immediately after rewarming from induced hypothermia, was decreased. Infusion of low molecular dextran during hypothermia prevented the occurrence of intravascular aggregation as well as the reduction of the tensile strength of the wounds. LÖFSTRÖM (1959a) showed that infusion of low molecular dextran during induction of hypothermia prevented the intravascular aggregation during hypothermia but did not effect the oxygen consumption during cooling or rewarming. Infusion of low molecular dextran after rewarming to 30—32° C resulted in an immediate increase in oxygen consumption, which was interpreted as a pay off on an oxygen debt.

LÖFSTRÖM (1959b) considered that in hypothermia down to 30° C the increased concentration of plasma proteins above a critical level and an increased concentration of erythrocytes are the main factors promoting intravascular aggregation. In hypothermia (20° C) a decreased pressure head and intravascular clotting provided the most likely causes of the pronounced intravascular aggregation and the stagnation of blood cells. He assigned the effect of infusion of low molecular dextran to the increase in the amount of low molecular substances in the blood, which increases the suspension stability of the blood.

The possibility that intravascular aggregation might to some extent obstruct the passage of the injected contrast medium through the capillaries of the brain in hypothermia in the present experiments could not be excluded. In order to test the significance of intravascular aggregation in this kind of experiments it was decided to carry out BBB experiments in hypothermia, in which the animals had been treated with infusion of low molecular dextran during the cooling according to LÖFSTRÖM & ZEDERFELDT (1957b).

Methods

The capillary circulation in the bulbar conjunctiva was assessed before the cooling (exceptions C 134, C 135, C 136, C 149) and in hypothermia before and after the ligation of the external jugular veins by means of a one-tube microscope from which the stage, the condenser and the illuminating mirror had been removed. The light was supplied by a spalt lamp provided with a heat absorbing filter. The microscope had 2 objectives with the data 3.5:1 and 10:1, $t=0.25$, respectively. The ocular was $\times 10$. The status of the conjunctival circulation was assessed with regard to the occurrence of aggregates and the velocity of flow (when aggregates occurred).

Two dextran solutions supplied from AB Pharmacia, Uppsala, Sweden, were used: 1. Dextran 6 % in saline denoted XDR 401 with an intrinsic viscosity of 0.16 at 20° C and a mean molecular weight of about 26 000. 2. Dextran 10 % in saline denoted 823—325 (U3) with an intrinsic viscosity of 0.18 at 20° C and a mean molecular weight of about 41 000. The dextran solutions were injected into a polyethylen catheter inserted into a femoral vein according to the following scheme adopted from LÖFSTRÖM & ZEDERFELDT (1957b): 20 ml at a body temperature of 32° C and 10 ml at body temperatures 28, 25, 23 and 21° C, respectively. Before the BBB experiment another 10 ml was injected.

In the BBB experiment Umbradil 50 % of room temperature was injected into the left common carotid artery with an application time of 20 seconds and an injection pressure of 200 mm Hg. The brain was cut, 3 sections being made from 4 parts of the brain. The experiments are described according to the concentration of the dextran solution given and the length of the action of the trpb.

Results

Treatment with low molecular dextran 6 %

The experimental data are given in tables 80 and 81. Three rabbits were cooled. The cooling time in water ranged from 66 to 92 minutes. The rectal temperature at the end of the cooling was 21.0° C. The body temperature at the injection of Umbradil ranged from 20.8 to 20.2° C. The injection was made on an average 120 minutes after the induction of cooling. Towards the end of the experiment the body temperature rose about 0.5° C.

The decided application time of 20 seconds was regularly achieved. The amount of injected Umbradil ranged from 38 to 40 ml. The injection time was about 23 seconds. The blood pressure before the injection was on an average 37 mm Hg. Only slight changes occurred in the blood pressure during the injection, the highest rise being 8 mm Hg. In C 134 the blood pressure fell to low levels during the trpb infusion. In the 2 others a slight fall occurred, followed in one of them by a rise to the pre-injection level. No convulsions appeared. The trpb solution was infused in about 7 minutes. The animals were all alive 30 minutes after the beginning of the infusion of the trpb solution and were then killed.

Macroscopic examination. — The areas normally permeable to trpb were stained blue. The dura mater was stained blue, in one experiment (C 134) the staining was patchy. As seen from table 81 the brain was negative in 2 experiments and slightly stained in one. The staining was confined to the middle part of the pyriform lobe and the adjacent temporal

lobe on the injected side. On the surface the staining tended to be confluent though scattered, pin-point large, more intense areas appeared. On the cut surface the streaky staining was seen in the cortex beneath the stained surface.

Microscopic examination. — In C 136 the walls of the vessels were diffusely stained and surrounded by a faint staining of the parenchyma. Occasionally parenchymal cell nuclei were stained. In other areas as in the two other brains no staining of the parenchyma or the vessel wall occurred.

Treatment with low molecular dextran 10 %

Trypan blue duration about 10 minutes

Three rabbits, treated with dextran 10 % during cooling and then submitted to BBB experiments, died about 10 minutes after the beginning of the trpb infusion. The experimental data are given in tables 82 and 83. The cooling time in water ranged from 60 to 95 minutes. The rectal temperature at the end of cooling in water was about 21.0° C. The body temperature at the injection of Umbradil ranged from 20.5° to 20.9° C and remained unchanged until the end of the experiment. The decided application time of 20 seconds was achieved. The time for the return of the blood to the pial vessels after the end of the injection varied from 5 to 15 seconds. The amount of injected Umbradil was about 30 ml. The injection time was about 23 seconds. The blood pressure before the injection varied from 15 to 60 mm Hg. Only slight changes occurred during the injection procedure, the highest rise being 10 mm Hg. The blood pressure fell below 10 mm Hg during the later part of the trpb infusion or just after the end and the pulse ceased. The trpb solution was infused in about 7 minutes. No convulsions occurred.

Macroscopic examination. — The areas normally permeable to trpb were stained blue with the exception of the hypophysis of C 158. The median eminence in this experiment was faintly blue. The dura mater was blue in C 149 but faintly blue in the 2 others, particularly in C 158. A few vessels on the surface of the injected hemisphere were blue in C 149 and C 158. Otherwise the brains were unstained on the surface and the cut surface (table 83).

Microscopic examination. — No staining of the brain parenchyma was seen. In all brains occasional staining of the vessel wall in vessels larger than capillaries occurred. In 2 of the brains it was almost exclusively confined to the cerebrum on the injected side. The staining was diffuse even though exceptional staining of the endothelial nuclei occurred. The choroid plexuses were stained in the vessel wall and in one brain also in the epithelium.

Trypan blue duration 20-30 minutes

The experiments were performed on 8 rabbits. The experimental data are given in tables 84 and 85. The rectal temperature at the end of the cooling was 21.0°C . The body temperature at the injection of Umbradil had a mean of 20.4°C . A slight rise, at most 0.4°C , occurred towards the end of the experiment.

The decided application time of 20 seconds was achieved. The time for the return of blood in the pial vessels after the end of the injection was recorded in 7 experiments and ranged from 3 to 13 seconds. The amount of injected Umbradil ranged from 23 to 40 ml. The injection time was about 23 seconds.

The blood pressure before the injection varied from 20 to 50 mm Hg. The changes in blood pressure during the injection were slight, the highest rise being 10 mm Hg. As a rule the blood pressure was about 20 to 30 mm Hg during the trpb infusion. In the animals with a blood pressure less than 10 mm Hg at the end of the experiment the blood pressure fell during or after the end of the trpb infusion. At the end of the experiment the blood pressure was below 10 mm Hg in 4 experiments and varied in the other 4 from 15 to 20 mm Hg. The pulse at the end of the experiment was zero in 2 experiments and in one of them there were contractions of the heart when the thorax was opened. In the other 6 experiments the pulse rate ranged from 2 to 64. No convulsions occurred.

The trpb solution was infused in about 7 minutes. Two animals died about 20 minutes after the beginning of the trpb infusion and the other 6 were killed after 30 minutes. One of these 6 animals showed a decreasing pulse rate during the last half of the experiment. 1—2 beats per minute were recorded during the last minutes. On the opening of the thorax no contractions of the heart were visible.

Macroscopic examination. — The areas normally permeable to trpb were stained. The dura mater was stained blue with the exception of C 150, in which the dura mater was faintly stained. The staining was more pronounced on the right side in C 146, C 162 and C 163. The degree of the staining of the brain is given in table 85. The damaged brains were only faintly stained. The staining on the surface of the brain consisted of fine dots extending over the injected hemisphere and sometimes to the opposite parasagittal region, in C 153 also to the cerebellum. Patches more intensely stained than the rest of the damaged area could occur. The staining on the surface in C 163, which was negative on the cut surface, consisted of a fronto-parietal blue patch. In the left parieto-occipital region in C 147 a faintly blue staining and red blood appeared on the surface and was regarded as an artefact due to the trephination.

On the cut surface the staining was streaky or dotty and most pronounced in the injected internal carotid area. Staining on the right side and within the vertebral area occurred occasionally.

Microscopic examination. — The staining was as a rule confined to the vessel wall, which was diffusely stained particularly in vessels larger than capillaries. Staining of the wall of capillaries also occurred. In C 153 staining of the endothelial nuclei was seen. Only in C 164 was a slight but definite parenchymal staining observed. The staining occurred in small areas around the vessels, also those larger than capillaries. Exceptionally, staining of the vessel wall and diffuse parenchymal staining was noticed in vessels filled with red blood corpuscles. A very faint parenchymal staining was noticed in C 146 and C 153.

Trypan blue duration about 60 minutes

Five rabbits were used. The experimental data are given in tables 86 and 87. The rectal temperature at the end of the cooling was 21.0°C . The body temperature at the injection of Umbradil had a mean of 20.8°C and remained at that level to the end of the experiment. The decided application time of 20 seconds was achieved. The time for the refilling of the pial vessels with blood after the end of the injection varied from 2 to 7 seconds. The amount of injected Umbradil was on an average about 35 ml. The injection time was about 24 seconds. Before the injection the blood pressure varied from 20 to 65 mm Hg. During the injection procedure only slight changes in the blood pressure occurred, the highest rise being 6 mm Hg. In 3 animals the blood pressure during and after the trpb infusion was about 20 mm Hg. In C 151 and C 152 the blood pressure during the trpb infusion was 30—40 mm Hg, then in the former it varied between 30 and 60 mm Hg and in the latter rose to about 60 mm Hg and fell to about 20 mm Hg more than 30 minutes after the beginning of the trpb infusion. At the end of the experiment the blood pressure was low with the exception of one experiment. The pulse at the end of 2 experiments was very slow. The trpb solution was infused in 9 to 18 minutes. No convulsions occurred.

Macroscopic examination. — The areas normally permeable to trpb were stained blue as was also the dura mater. As seen from table 87 the staining of the damaged brains was faint and consisted of dots within the cerebrum on the left side. In C 152, which was considered negative on the surface, a few tiny, faintly blue dots occurred. The staining of the cut surface was streaky or dotty and faint. The damage was most pronounced in the cortex within the area of the internal carotid artery on the injected side, although blue staining could also occur within the vertebral area and the opposite internal carotid area.

Microscopic examination. — The macroscopically stained brains showed staining of intracerebral vessels down to the capillary level with a surrounding, faintly diffuse parenchymal staining. Exceptional staining of the vessel wall occurred in one of the negative brains. The median eminence, the epiphysis and the choroid plexuses were diffusely stained. The vessels were well rinsed even though trpb could remain in some of them.

Trypan blue duration 105—120 minutes

Five rabbits were used. The experimental data are given in tables 88 and 89. The rectal temperature at the end of the cooling was 21.0°C . The cooling time in water ranged from 50 to 70 minutes. The time from the beginning of the cooling to the time of injection varied from 95 to 115 minutes. The body temperature at the injection of Umbradil had a mean of 20.8°C and remained at that level to the end of the experiment.

The decided application time was achieved in all experiments but one, in which the vessels were refilled with blood during the injection after an application time of 18 seconds. In 4 experiments the time for the return of blood in the pial vessels after the end of the injection ranged from 3 to 6.5 seconds. The injected amount of Umbradil was on an average 33 ml, injected in about 22 seconds. The level of the blood pressure before the injection ranged from 30 to 70 mm Hg. During the injection procedure only slight changes in the blood pressure occurred, and the rise during the injection varied from 2 to 6 mm Hg. In C 157 and C 167 the blood pressure during the trpb infusion was about 50 mm Hg or more and kept a level of 40 to 50 mm Hg for at least the first hour after the beginning of the trpb infusion. In the others the blood pressure level was 20—30 mm Hg for the first hour. During the later part of the second hour the blood pressure was 10—25 mm Hg. At the end of the experiment the blood pressure was about 15 mm Hg and ranged from 0 to 25 mm Hg. The pulse at the end of the experiment was on an average 42 beats/min. In one experiment no pulse was visible but there were contractions of the heart on the opening of the thorax. The trpb solution was infused in about 8 minutes. No convulsions occurred. The animals were killed after 2 hours of trpb duration, except one which died after 1 hour and 45 minutes.

Macroscopic examination. — The areas normally permeable to trpb were stained blue. The dura mater was stained blue. In C 166 the left side was only faintly stained. The degree of the staining of the brain is given in table 89. On the surface the damage appeared as small patches

Table 20. *The conjunctival bulbar circulation assessed at normal body temperature and in hypothermia (20° C) after treatment with 10 % low molecular dextran and in untreated group in hypothermia (20° C). The figures denote number of animals.*

	Intravascular aggregates			Flow	
	None	Occurring occasionally	Occurring regularly	Fast	Slow
Treatment with low molecular dextran					
At 38° C ¹	7	13	0	13	0
At 20° C	2	10	9	17	2
Untreated group					
At 20° C	0	3	20	3	20

¹ One animal not examined at normal body temperature.

located within the injected side of C 160 and in the cerebellum of C 157. A trephination artefact occurred in C 166. A few tiny, faintly blue stained dots were seen on the injected side of C 167. The staining on the cut surface was faint and appeared as streaks and small patches. A staining visible only on the surface of the brain occurred in C 157. In the damaged brains the staining was generally seen within the area of the injected internal carotid artery but the other vascular territories could be stained to a smaller extent. In C 166 the most damaged area was the vertebral one.

Microscopic examination. — One of the brains was entirely negative. In the other 4 brains very slight staining of the parenchyma occurred. The walls of the vessels were stained to a varying extent and degree. It was felt that the extent and degree of the staining of the vessel wall was less pronounced than in the previous group (trpb duration 60 min.). In 3 brains (C 157, C 160 and C 167) a few scattered petechial hemorrhages occurred, surrounded by a blue brim. In C 157 such hemorrhages without surrounding staining was noticed. The choroid plexuses were stained in the vessel wall and, though faintly, in the epithelium. In 2 experiments staining of parenchymal nuclei was seen in the median eminence.

Assessment of conjunctival bulbar circulation

The capillary circulation in the bulbar conjunctiva was assessed at normal body temperature and in hypothermia before the injection of Umbradil. The results from the group treated with 10 per cent low molecular dextran are presented in table 20 together with a group not treated with low molecular dextran in which the bulbar conjunctival circulation was assessed in hypothermia 20° C (before preparation on

the neck). As seen from table 20 the capillary circulation appeared to be impaired in hypothermia also when low molecular dextran was given but not to such a degree as in the untreated group. The velocity of the flow, however, seemed to be almost unaltered in the group to which low molecular dextran had been administered, as compared with the status at normal body temperature.

The effect of noradrenaline on blood pressure in hypothermia and the influence of increased blood pressure at the injection on injury to blood-brain barrier

The possibility that the low blood pressure in hypothermia might be a factor of importance for decreasing the damage to the BBB could not be excluded in view of the following observations: One animal at a body temperature of 25° C had a degree of injury to the BBB compatible with that obtained in normothermic animals. The blood pressure before the injection was about 85 mm Hg. The same applies to the animal with the most damaged brain on injection of Umbradil 70 % at a body temperature of 20° C. This might have been due to chance but since two events coincided which did not regularly occur in hypothermia, it was felt necessary to investigate systematically the effect on the degree of damage of the blood pressure level at the injection.

If the filtration pressure is of importance for the passage of trpb into the brain parenchyma, a low blood pressure level during and after the trpb infusion might cause a diminished lesion. This will be elucidated further on, when the experiments with lowered blood pressure in normothermic animals will be compared with a group taken from the series of animals treated with low molecular dextran with a blood pressure level of the same order as the just mentioned animals of normal body temperature.

It was decided to use noradrenaline, which MOYER, MORRIS & DE BAKEY (1956) reported to raise the blood pressure in dogs with a body temperature of about 25° C to normothermic levels. In order to obtain a control group, the effect of noradrenaline was tested under the present experimental conditions without injection of Umbradil. This series was also used for a study of the relationship between the brain temperature and the rectal temperature during cooling.

1. Infusion of noradrenaline in saline and in trypan blue solution and measurements of brain and rectal temperature

Experimental. — Five rabbits were cooled. A burr hole was made on one side and the brain temperature recorded with the needle formed applicator of the electric thermometer. The rectal temperature was

measured 9 cm above the anus. The brain temperature was measured at the same time as the rectal temperature, every 15 minutes. The needle applicator was inserted to just above the base of the skull. The cooling water was turned off during the temperature recording. After the end of the cooling the animals were placed on the operating table and noradrenaline (Nor-exadrin, Astra, 10 μ g solved in 100 ml saline) infused at a rate of about 0.2 μ g/kg body wt/min. In 2 experiments saline only was infused at the same rate as the noradrenaline solution. In 2 other experiments the noradrenaline was given in 100 ml 0.5 per cent trpb solution. In these 2 experiments the brain was removed after rinsing of the vessels and cut, 3 sections being made from 4 parts of the brain.

Results

The experimental data are given in tables 90 and 91. The rectal temperature at the end of cooling had a mean of 21.2° C. The cooling time in water varied from 60 to 100 minutes. The time from the beginning of the cooling to the start of the infusion varied from 91 to 138 minutes.

After the start of the infusion of noradrenaline in saline the blood pressure increased from an initial level with a mean of about 35 mm Hg (range 20—60 mm Hg) to a level of about 85 mm Hg (range 70—95 mm Hg) in about 5 minutes, which was kept for the rest of the infusion. The rise in blood pressure from the start of the infusion of noradrenaline varied from 25 to 55 mm Hg with a mean of about 40 mm Hg.

In the 2 experiments with infusion of noradrenaline solved in trpb the course of events was somewhat different. In C 257 the blood pressure rose from 30 to 90 mm Hg in 4 to 5 minutes. After about 10 minutes on this level the blood pressure fell about 40 mm Hg. Addition of 2 μ g of noradrenaline to the infusion restored the previous level of the blood pressure. After 2 minutes the trpb solution was used up and replaced by noradrenaline solved in saline. The blood pressure kept at a level of about 90 mm Hg for 7 minutes and then fell to about 75 mm Hg. As the trpb infusion was used up earlier than calculated, the dose of noradrenaline was higher than 0.2 μ g/kg/min. In C 258 the blood pressure rose to only 30 mm Hg in 5 minutes with a dose of noradrenaline of about 0.2 μ g/kg/min. The dose was about doubled by addition of 10 μ g of noradrenaline to the solution, which caused a rise in blood pressure to about 85 mm Hg in 2 minutes. After 4 minutes the blood pressure dropped to about 40 mm Hg and was not restored although more noradrenaline was added to the infusion. The infusion of saline caused only a slight increase in the blood pressure (see table 91).

Macroscopic examination. — Only the brains of the trpb infused animals

were removed and showed subarachnoid hemorrhage. On the sections the walls of the needle track were stained blue, otherwise the sections were negative. The dura mater and the areas normally permeable to trpb were stained blue.

Microscopic examination. — The microscopic investigation showed that the needle track was filled with blood. The surrounding parts of the parenchyma showed diffuse blue staining and staining of parenchymal nuclei. Otherwise the parenchyma was unstained. The lumen of a few capillaries in the cortex contained trpb. The vessel wall was not stained. The base of the brain was covered with blood which was not blue.

Comparison of brain and rectal temperatures. — The results from the temperature measurements are presented in table 21. There was a close agreement between the brain temperature and the rectal temperature.

Conclusion. — Infusion of noradrenaline in hypothermic rabbits caused a rise of the blood pressure to a level corresponding to the one at normal body temperature. When noradrenaline was solved in the trpb solution the blood pressure rise was obtained but it seemed as if the dose had to be higher. The increased blood pressure did not by itself cause any damage to the BBB. The rectal temperature measured 9 cm above the anus showed close agreement with the brain temperature during and after cooling.

2. Injection of Umbradil 50 % of room temperature

Experimental. — Five rabbits were cooled to a body temperature of about 20° C and then prepared for the BBB experiment. Noradrenaline was infused (about 0.2 µg/kg body wt/min) before and during the injection of Umbradil. Umbradil 50 % of room temperature was injected into the left common carotid artery with an application time of 20 seconds. Noradrenaline (10 µg) was added to the trpb solution. In the experiments with a high infusion rate of trpb the dose of noradrenaline was doubled or quadrupled. The brain was cut, 3 sections being made from 4 parts of the brain.

Results

The experimental data are given in tables 92 and 93. The rectal temperature at the end of the cooling had a mean of 21.4° C. The time for cooling in water ranged from 50 to 85 minutes. The time from the beginning of cooling to the injection ranged from 92 to 127 minutes.

The body temperature at the injection was on an average 21.1° C and rose about 0.5° C towards the end of the experiment. The amount of injected Umbradil varied from 28 to 38 ml. The injection time varied

Table 21. Brain and rectal temperatures in °C during cooling to a body temperature of about 20° C and during infusion of noradrenaline.

Rabbit No	C 243			C 248			C 253			C 257			C 258		
	Temperature		Difference rectal and brain temp.	Temperature		Difference rectal and brain temp.	Temperature		Difference rectal and brain temp.	Temperature		Difference rectal and brain temp.	Temperature		Difference rectal and brain temp.
	Rectal	Brain		Rectal	Brain		Rectal	Brain		Rectal	Brain		Rectal	Brain	
Before cooling	38.0	37.7	0.3	38.1	—	—	38.0	37.8	0.2	37.4	37.4	0	37.6	37.4	0.2
15 min. in water	33.6	33.2	0.4	35.0	35.4	-0.4	32.8	32.8	0	30.8	31.0	-0.2	33.3	32.8	0.5
30 "	30.0	29.2	0.8	32.8	32.4	0.4	28.4	28.2	0.2	26.9	26.1	0.8	29.2	28.6	0.6
45 "	27.0	26.4	0.6	29.2	29.4	-0.2	26.4	25.8	0.6	23.4	23.1	0.3	26.0	25.3	0.7
60 "	24.2	23.8	0.4	26.7	26.7	0	23.0	23.0	0				22.9	22.6	0.3
75 "				24.2	24.8	-0.6	21.2	21.4	-0.2				21.6	20.2	1.4
90 "				22.6	22.9	-0.3									
105 "				21.6	22.1	0.5									
End of cooling	22.0	21.9	0.1	20.8	21.7	-0.9				20.9	20.5	0.4	20.6	19.8	0.8
Before infusion	21.8	21.7	0.1	20.4	21.0	-0.6	20.3	20.5	-0.2	19.4	18.8	0.6	19.8	18.4	1.4
During infusion	21.8	21.8	0	21.0	20.5	0.5	19.8	19.6	0.2	19.5	19.0	0.5	20.0	18.6	1.4
				21.0	20.4	0.6	20.0	19.6	0.4						
Mean			0.3			-0.1			0.1			0.3			0.8

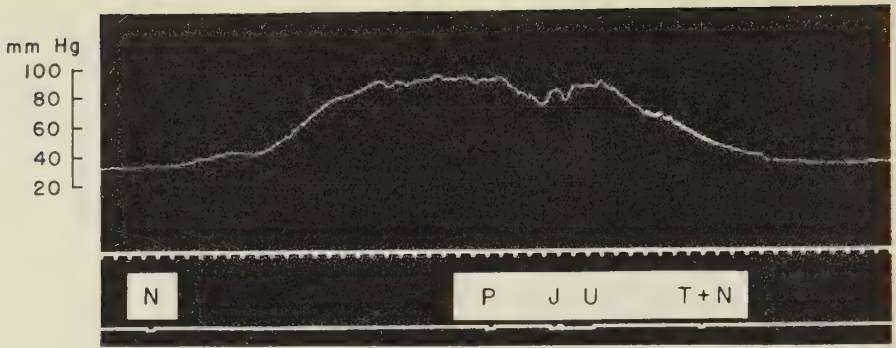


Fig. 5. Hypothermia (20°C). Exp. C 282. Injection of Umbradil 50 % of room temperature into common carotid artery after increase in blood pressure by means of noradrenaline. Umbradil 35 ml. Inj. time 21 sec. N: Infusion of noradrenaline $0.2\text{ }\mu\text{g/kg}$ body wt/min. P: Pilot injection with saline. J: Removal of jugular clamps. U: Injection of Umbradil. T+N: Infusion of noradrenaline solved in trpb $0.2\text{ }\mu\text{g/kg}$ body wt/min. The jugular clamps are replaced between U and T. Time marking: 10 sec. Note absence of blood pressure rise during injection.

from 21 to 26 seconds. The decided application time of 20 seconds was achieved. The time for the refilling of the pial vessels with blood after the end of the injection varied from 1 to 8 seconds. Before the infusion of noradrenaline the blood pressure varied from 20 to 65 mm Hg (mean about 35 mm Hg). The rise in blood pressure during the infusion of noradrenaline was on an average about 45 mm Hg. As a rule, only slight changes occurred in the blood pressure during the injection of Umbradil, the highest rise being 18 mm Hg. In 2 experiments a slight fall in the blood pressure took place. The blood pressure after the injection was on an average about 45 mm Hg (range 30—80 mm Hg). During the first part of the trpb infusion the blood pressure was 30—50 mm Hg and later fell below 20 mm Hg, even when the dose of noradrenaline was considerably increased (fig 5).

The pulse was visible only in one animal at the end of the experiment. In 2 others there were contractions of the heart on the opening of the thorax. In the other 2 animals the heart ceased to beat 13 and 21 minutes after the beginning of the trpb infusion, respectively. In all experiments the rinsing was not made until 30 minutes after the beginning of the trpb infusion.

Macroscopic examination. — The areas normally permeable to trpb were stained blue with the exception of the hypophysis of C 279. The dura mater was stained blue though only faintly in C 282. In C 284 the dura mater was stained only on the right side and in C 286 the staining was more pronounced on the right side.

The degree of injury is given in table 93. The damage was slight. The staining on the surface consisted of tiny dots or points. The cut surface of the damaged brains was streaky or dotted. In C 286 a faint staining occurred in the ventral part of the hippocampus. In C 280 the damage was seen within all 3 vascular territories with no difference in degree. The medulla oblongata, however, was not stained. In C 284 the lesion was confined to the left cortex.

Microscopic examination. — The 2 brains judged as slightly damaged on the cut surface showed a slight, diffuse staining of the parenchyma around the capillaries, the walls of which were diffusely stained. In these 2 brains and in two others the vessel wall was seen to be diffusely stained to a varying extent. Only a few vessels of C 286 were stained. No extravasal staining was seen in the ventral part of the left hippocampus. The vessels were well rinsed. The choroid plexuses were stained in the vessel wall and diffusely in the epithelium. The median eminence was stained a diffuse blue and in C 279, C 282 and C 286 staining of the parenchymal nuclei also occurred. The wall of the pericallosal arteries was not stained.

II. INJECTION INTO COMMON CAROTID ARTERY WITH LIGATION OF EXTERNAL CAROTID ARTERY

A possible reason for the decrease of the injury to the BBB in hypothermia (20° C) might be that a larger bulk of the injected contrast medium passed into the external carotid artery in hypothermia than in normothermia. The occurrence of intravascular aggregation and the absence of arterio-venous anastomoses in the brain might facilitate such a redistribution of the injected medium owing to a relatively lower resistance to the flow of the contrast medium into the external carotid artery. True, the experiments with low molecular dextran did not support such an assumption, but owing to the critical importance of such a redistribution it was necessary to test the effect of ligation of the external carotid artery in hypothermia.

Therefore experiments with Umbradil 50 % with an application time of 20 and 40 seconds were performed. As will be shown, the lesion was still less pronounced than in normothermia. In order to study the possibility of producing a lesion in hypothermia of the same degree as in normothermia, Umbradil 70 % was injected with increasing application times.

Umbradil 70 % of 38° C

Experimental. — Five animals were cooled to a body temperature of about 20° C. After preparation with ligation of the left external carotid

artery Umbradil 70 % heated to about 38° C was injected into the left common carotid artery. The animal's head was turned to the left. The application time varied from 40 to 150 seconds. The animals were killed 30 or 15 minutes after the beginning of the trpb infusion. The brain was cut, 3 sections being made from 4 parts of the brain.

Results

The experimental data are presented in tables 94 and 95. At the end of the cooling the body temperature was about 21° C. The cooling time in water varied from 45 to 55 minutes. The time from the beginning of the cooling to the injection of Umbradil varied from 88 to 110 minutes. At the time of injection the body temperature was on an average 20.4° C and rose about 0.5° C towards the end of the experiment. The decided application time was achieved. The time for the refilling of the pial vessels with blood after the end of the injection varied from 7 to 30 seconds. The highest value was obtained in the experiment with the longest application time and the lowest blood pressure. The injected amount did not regularly increase with the length of application time but was largest in the experiments with an application time of 150 seconds. The blood pressure before the injection varied from 10 to 45 mm Hg. The changes during the injection procedure were slight, the highest rise being 8 mm Hg. During the infusion of trpb the blood pressure varied from 15 to 30 mm Hg and fell to about 10 mm Hg towards the end of the experiment. The trpb solution was infused in about 8 minutes. In these experiments there were definite contractions of the heart on the opening of the thorax. No convulsions occurred.

Macroscopic examination. — The choroid plexuses, the median eminence, the epiphysis and the area postrema were stained blue in all experiments. The hypophysis was stained in C 225 and faintly in C 220. It was not stained in the other experiments. The staining of the dura mater was faint in all experiments.

The degree of the injury is given in table 95. It occurred that the staining was more intense on the surface than on the cut surface. The staining on the surface was most pronounced around the vessels and was only confluent in the right hemisphere of C 223. On the cut surface the staining was streaky or dotted. The staining was most pronounced in the right side of the cerebrum of C 223, and showed a tendency to be confluent. In C 218, C 223 and C 225 the pia mater stood out as a markedly stained blue brim around the parenchyma.

No area was more damaged than the injected internal carotid area.

Microscopic examination. — The microscopic picture was the same as

in the series with injection of Umbradil 70 % into the common carotid artery. Thus the staining of the vessel wall was very pronounced with staining of the endothelial nuclei down to the capillary level in mostly well rinsed vessels. The pia mater was stained intensely blue and stood out as a blue brim surrounding the brain in 3 experiments. The staining of the parenchyma was most intense in C 223 with the longest application time: In addition to the stasis a diffuse halo and staining of the parenchymal nuclei were seen. In this as in the other experiments a pronounced staining of the vessel wall was often seen without any blueness of the parenchyma.

In C 220 the walls of the vessels were stained and the rinsing was defective. No parenchymal staining was seen.

Sometimes a diffuse staining of the parenchyma was seen nearest to the pia mater. With the exception of C 225, the choroid plexuses showed stasis with no staining of the vessel wall or the epithelium on the left side even though stasis was not seen in other parts of the left side of the cerebrum. On the right side the choroid plexuses were stained in the endothelial nuclei and often also in the epithelial nuclei. In C 225 the staining was diffusely blue on both sides and in C 223 the choroid plexuses on the right side also showed stasis and faint staining of the epithelium.

Umbradil 50 % of room temperature

1. Application time 40 seconds

Experimental. — Five animals were cooled to a body temperature of about 20° C and then prepared for the BBB experiment. Umbradil 50 % of room temperature was injected into the left common carotid artery, the external carotid artery being ligated, with an application time of 40 seconds. The intention was to kill the animals 30 minutes after the injection. The brain was cut, 3 sections being made from 4 parts of the brain.

Results

The experimental data are given in tables 96 and 97. The rectal temperature at the end of the cooling in water was about 21° C. The cooling time in water varied from 40 to 67 minutes. The time from the beginning of the cooling to the injection varied from 85 to 110 minutes. The body temperature at the injection had a mean of 20.4° C and was virtually unchanged to the end of the experiment. The decided application time was achieved. The time for the refilling of the pial vessels with blood after the end of the injection varied from 4 to 6 seconds. The amount

of injected Umbradil ranged from 5 to 12 ml. The injection time varied from 41 to 42.5 seconds.

The blood pressure before the injection was on an average about 30 mm Hg. The rise in blood pressure during the injection was on an average about 8 mm Hg (range 2—18 mm Hg). During the infusion of trpb the blood pressure was about 20 mm Hg and fell below 10 mm Hg towards the end of the experiment. The pulse was hardly discernible at the end of the experiment. However, there were definite contractions of the heart on the opening of the thorax with the exception of C 185, which was alive 30 minutes after the injection but then died.

The trpb infusion was completed in 6—9 minutes. In 3 of the experiments the animals died about 30 minutes after the beginning of the trpb infusion, and in the 2 others about 10 minutes after the beginning of the trpb infusion. No convulsions occurred.

Macroscopic examination. — The choroid plexuses, the median eminence, the epiphysis and the area postrema were stained blue. The hypophysis was stained in C 185, faintly stained in C 187 and C 193 and unstained in C 191 and C 189. The staining of the dura mater was faint in all experiments.

The degree of the injury is given in table 97. In C 191 and C 193 the staining on the surface was confluent. In C 185 it was dotty. The damage was confined to the left hemisphere. In C 189 a faint staining of the left hippocampus occurred.

On the cut surface the staining was streaky or dotty, but areas with confluent staining were found in C 191 and C 193. The damage was most pronounced within the area of the injected internal carotid artery. The cerebellum was not damaged.

Microscopic examination. — In all experiments, the walls of the intracerebral vessels were stained to a varying extent. In 3 of them (C 191, C 193, C 189) the nuclei of the endothelial cells were occasionally stained. Staining of the parenchyma occurred in 4 brains and was most pronounced in C 191 and C 193, in which staining of the parenchymal nuclei was seen. There was also stasis within a small area in C 193. The choroid plexuses were stained in the epithelium and occasionally also in the nuclei, most markedly so in C 193. The vessels were well rinsed with the exception of C 185.

2. Application time 20 seconds

Experimental. — Five animals were cooled to a body temperature of about 20° C and prepared for the BBB experiment. Umbradil 50 % of room temperature was injected into the left common carotid artery after ligation of the external carotid artery with an application time of 20

seconds. The intention was to kill the animals about 30 minutes after the injection of Umbradil. The brain was cut, 3 sections being made from 4 parts of the brain.

Results

The experimental data are given in tables 98 and 99. The rectal temperature at the end of the cooling was 21.0°C . The cooling time in water varied from 50 to 72 minutes. The time from the beginning of the cooling to the injection ranged from 93 to 118 minutes.

The body temperature at the injection of Umbradil was 20°C and rose about 0.5°C towards the end of the experiment. The decided application time of 20 seconds was achieved. The amount of injected Umbradil varied from 3 to 5 ml and was injected in about 22 seconds. The time for refilling of the pial vessels with blood after the end of the injection varied from 4 to 14 seconds.

The mean value of the blood pressure before the injection was about 25 mm Hg. The rise in blood pressure during the injection of Umbradil was 4 mm Hg at most. During the infusion of trpb the blood pressure was 20—40 mm Hg. At the end of the experiment the blood pressure was 80 mm Hg in one experiment and about 10 mm Hg in the other 4.

The trpb solution was infused in 5—30 minutes. The animals lived from 16—35 minutes after the injection. No convulsions occurred.

Macroscopic examination. — The choroid plexuses, the median eminence, the epiphysis, the hypophysis and the area postrema were stained blue. The hypophysis, however, was not stained in C 179 and the area postrema was faintly stained in C 174. The dura mater was faintly stained in C 179. In C 181 and C 183, the dura mater on the left side was faintly stained.

On the surface of C 174 an area smaller than an olfactory bulb was stained in the fronto-parietal region on the left side. In C 183 a few vessels in the left frontal region were stained.

On the cut surface a faint streaky staining was seen in part of the cortex, hippocampus and the thalamus on the left side in C 183.

Microscopic examination. — The parenchymal staining observed in C 181 and C 183 was very faint and on rare occasions occurred as a diffuse staining around vessels in the left cortex. The small stained area in C 174 showed staining of the vessel wall, surrounded by staining of the parenchymal nuclei. The lumen of the capillaries in the area contained trpb. The staining of the vessel wall was rather an exception and the walls of most of the vessels were unstained. The choroid plexuses of the epithelial nuclei were also seen to be stained. The wall of the pericallosal

artery was not stained. In C 177, C 181 and C 183 the rinsing of the vessels was poor.

C. COMPARISON OF SERIES IN HYPOTHERMIA

The cooling data for hypothermia, 25° C and 20° C, respectively, are reviewed in tables 22 and 23. It is seen that an increase in water temperature from about 12° C to about 24° C more than doubled the cooling time in water on cooling to a body temperature of 25° C. The water temperature was about 2° C higher in the series with cooling to a body temperature of 25° C than in that with cooling to 20° C. The series including treatment with low molecular dextran 10 % cooled on an average 10 minutes more slowly than the group with ordinary cooling to a body temperature of 20° C (table 23). This was probably due to the higher body weight in the dextran group. It is seen from table 24 that the cooling time increases with body weight. With regard to the body weight, the cooling time is still about 5 minutes longer in the dextran group in those 2 body weight groups which include the largest number of animals. However, the difference between the mean values is not significant on comparison according to the Mann-Whitney U test. In table 25 it is shown that the variations in water temperature during ordinary cooling did not influence the length of cooling time with the exception of a water temperature between 12 and 12.9° C, in which group the cooling time was longer than in the others. However, the body weight happens to be greater in this group, which contributes to a longer cooling time.

The body temperature was 24.6 ± 0.9 (S.D.)° C in the group denoted hypothermia (25° C) for 31 animals and 20.5 ± 0.6 (S.D.)° C for 75 animals in the group denoted hypothermia (20° C). The body temperature in the 21 animals treated with low molecular dextran 10 % was 20.6 ± 0.3 (S.D.)° C.

In the different series in hypothermia (25° C) the body temperature was of about the same magnitude as was the volume of injected Umbra-dil. The volume of saline in the control series was somewhat larger. The injection time was of the same magnitude. In all series there was incomplete expelling of the blood from the pial vessels since, during part of the injection, the blood in the veins was not regularly expelled. The length of the infusion of trpb varied much in the various series but was generally about 10 minutes. In all series it occurred that animals died before the decided length of trpb duration, 30 minutes, was up, even in the experiments with injection of saline. The pulse rate before the injection was about 100 beats per min. and the blood pressure 40—50 mm Hg. The series with prolonged cooling showed a lower pulse rate

Table 22. Cooling data for the series in hypothermia (25° C). Figures in brackets denote number of animals.

	Body wt kg	Body temperature °C						Cooling time in water min.	Water temp. °C
		before cooling	after cooling in				end of cooling		
			15 min.	30 min.	60 min.	90 min.			
Ordinary cooling									
Mean ± S.D.	2.0 ± 0.6 (25)	38.3 ± 0.6 (25)	32.1 ± 1.3 (20)	28.4 ± 2.1 (25)	28.0 (1)		25.9 ± 1.1 (20)	45 ± 12 (24)	11.9 ± 1.1 (25)
Slow cooling									
Mean ± S.D.	1.9 ± 0.2 (6)	37.7 ± 0.8 (6)		32.4 ± 0.6 (6)	29.1 ± 1.1 (6)	28.1 ± 1.2 (5)	26.3 ± 0.7 (3)	26.7 ± 0.7 (6)	23.8 ± 1.6 (6)

Table 23. Cooling data for the series in hypothermia (20° C). Figures in brackets denote number of animals.

	Body wt kg	Body temperature °C						Cooling time in water min.	Water temp °C
		before cooling	after cooling in			end of cooling			
			30 min.	60 min.	90 min.				
Ordinary cooling									
Mean ± S. D.	2.0 ± 0.3 (75)	38.0 ± 0.5 (75)	28.1 ± 2.6 (75)	23.7 ± 1.9 (45)	22.7 ± 1.2 (9)	21.3 ± 0.5 (75)	66 ± 19 (75)	9.4 ± 1.2 (75)	
Cooling with treatment with low molecular dextran 10 %									
Mean ± S. D.	2.2 ± 0.4 (21)	37.4 ± 0.7 (21)	29.6 ± 2.3 (21)	24.0 ± 2.2 (17)	22.5 ± 1.1 (4)	21.0 ± 0.2 (21)	76 ± 18 (21)	9.8 ± 0.5 (21)	

Table 24. Comparison of body weight and cooling time in water in hypothermia (20° C) in the series with ordinary cooling and cooling with pretreatment with low molecular dextran 10 %.

Ordinary cooling								
Body wt kg	1.4—1.5	1.6—1.7	1.8—1.9	2.0—2.1	2.2—2.3	2.4—2.5	2.6—	
Number of animals	3	13	23	15	12	6	3	
Cooling time min.								
Mean, range	53, 45—58	56, 40—77	55, 35—78	67, 43—100	78, 40—105	86, 70—100	105, 82—133	
Cooling with treatment with low molecular dextran 10 %								
Body wt kg	1.6—1.7	1.8—1.9	2.0—2.1	2.2—2.3	2.4—2.5	2.6—2.7	2.8—	
Number of animals								
Cooling time min.	1	5	6	2	1	4	2	
Mean, range	50	60, 55—75	71, 60—84	79, 78—80	80	97, 85—113	99, 82—115	

Mann-Whitney U test		
Subject of test	U	P
Cooling time in the 2 cooling procedures in body wt group 1.8—1.9	42.5	>0.05
in body wt group 2.0—2.1	39	>0.05

Table 25. *Comparison of water temperature and cooling time in hypothermia (20° C). Ordinary cooling.*

Water temperature ° C	7.0—7.9	8.0—8.9	9.0—9.9	10.0—10.9	11.0—11.9	12.0—12.9
Number of animals	1	26	31	6	5	6
Cooling time min.						
Mean, range	43	68, 37—100	60, 35—100	65, 50—85	69, 45—105	86, 65—133
Body wt kg.						
Mean $\pm$ S. D.	1.9	2.1 $\pm$ 0.2	1.9 $\pm$ 0.4	2.0 $\pm$ 0.1	1.9 $\pm$ 0.2	2.2 $\pm$ 0.3

and a lower blood pressure than the series with normal cooling and slow cooling. In table 26 it is shown that the difference in pulse rate between the 3 groups is significant on the 2 per cent level. As seen from the mean ranks it is the group with the prolonged cooling alone that contributes to the difference. A tendency to difference in the blood pressure before the injection between the 3 groups was found which is also due to the group with prolonged cooling.

At the end of the experiment the blood pressure was very low, often below 10 mm Hg, and in about a third of the animals the heart had stopped beating at the end of the experiment.

The cooling time at the onset of the BBB experiment amounted on an average to about 80 minutes on ordinary cooling and about 3 hours in the other groups.

The experimental data for the various series in hypothermia (20° C) showed that the body temperature in the BBB experiment was of about the same magnitude in all series, but was on an average 1° C higher in

Table 26. *Pulse rate and blood pressure before injection in the 3 groups with varying cooling procedure in hypothermia (25° C) tested with the Kruskal-Wallis one-way analysis of variance by ranks.*

	Ordinary cooling	Prolonged cooling	Slow cooling	H corr. ties	P
Pulse rate					
Number of animals	13	5	6		
Sum of ranks	198.5	24.5	77.0	7.82	<0.02
Mean of ranks	15.3	4.9	12.8		
Blood pressure					
Number of animals	10	5	6		
Sum of ranks	120.5	27.0	83.5	5.76	<0.10
Mean of ranks	12.1	5.4	13.9		

the series with infusion of noradrenaline. In the series with injection into the common carotid artery the amounts of injected Umbradil was of the same magnitude in comparable series but tended to be larger at injection of Umbradil 50 % of 38° C. The amount of saline injected in the control series was larger than the amount of Umbradil in comparable series. The largest volume of Umbradil 70 % of 38° C was smaller than the smallest volume of Umbradil 50 % of 38° C (Mann-Whitney U test: $P < 0.01$).

The injection time was of the same magnitude in comparable series. The time for the refilling of the pial vessels with blood after the end of the injection seemed to be longer in the series with lower blood pressure during the injection. Such a tendency was also noted in the experiments within the series, even if exceptions occurred. On injection of saline into the common carotid artery with an application time of 20 seconds the time for refilling of the pial vessels with blood after the end of the injection was about the same or somewhat shorter than on injection of Umbradil.

Incomplete expelling of the blood in the pial vessels occurred within all series at injection into the common carotid artery in about half of the experiments and in one instance at injection with ligation of the external carotid artery. The incompleteness was due to defect venous filling with 2 exceptions, both occurring in the series with injection of Umbradil 50 % of room temperature into the common carotid artery with an application time of 20 seconds. For part of the application time in this series, the blood in the arteries was only intermittently expelled.

In all series, even on injection of saline and on injection with ligation of the external carotid artery, animals succumbed before the decided length of the trpb duration was up. This occurred in about half of the animals on normal cooling and in about a quarter on cooling with simultaneous treatment with low molecular dextran.

Variations were made in the length of the infusion of trpb during the experiments in order to evaluate whether the rate with which the trpb solution was infused exerted any influence upon the length of the survival of the animals. From the experiments within the series it is seen that the rate of infusion of trpb solution did not influence the length of survival.

The blood pressure before the injection was higher in the dextran treated group than in the others, and statistical comparison with animals otherwise treated in a similar way showed that the difference of about 10 mm Hg was significant on the 2 per cent level (table 27). Within the dextran treated group the blood pressure before the injection seemed to be somewhat higher in the series with a longer survival time.

Table 27. Comparison of pulse rate per minute and blood pressure before injection in animals treated with low molecular dextran 10 % and an untreated group (series presented in tables 39, 46, 49, 73, 75, 77 and 79). Figures in brackets denote number of animals. Statistical treatment with *t*-test.

	Pulse rate per min. Mean $\pm$ S.D.	Blood pressure mm Hg Mean $\pm$ S.D.
Low molecular dextran group	65 $\pm$ 10 (21)	42 $\pm$ 15 (21)
Untreated group	52 $\pm$ 16 (36)	31 $\pm$ 17 (35)
Difference $\pm$ S.E.	13 $\pm$ 3.9	11 $\pm$ 4.4
Degrees of freedom	55	54
P	<0.01	<0.02

The pulse rate per minute before the injection was about 50 in the animals cooled in the normal way but above 60 in animals treated with infusion of low molecular dextran during cooling. The difference was significant (table 27). In the dextran treated group the pulse rates before injection did not differ between the series. Within the series the animals with a higher pulse rate before the injection did not tend to survive longer.

At the end of the experiment the blood pressure was very low, often below 10 mm Hg. A relatively high blood pressure during the action of trpb seemed to occur more often in the animals treated with low molecular dextran.

As a rule a rise in blood pressure during the injection occurred but was fairly small in all series. The rise tended to be higher in the 2 series with injection of Umbradil of 38° C. The rise was on an average lowest at injection of Umbradil into the common carotid artery with ligation of the external carotid artery with an application time of 20 seconds.

On comparison of the series with hypothermia (25° C) and hypothermia (20° C) it was seen that the amount of injected Umbradil was about the same. The amount of saline used in the control series was also of about the same magnitude. The injection time in comparable series showed no difference. The frequency of incomplete expelling seemed to be lower in hypothermia (20° C) than in hypothermia (25° C). The proportion of animals surviving up to 30 minutes was not higher in the series with a body temperature of 25° C.

As might be expected, the pulse rate was higher in hypothermia (25° C). As a rule, the blood pressure was also higher. As compared with the animals treated with low molecular dextran, however, the difference in blood pressure was negligible.

In the experiments at a body temperature of 25° C only the median eminence, the choroid plexuses, and the area postrema were examined.

These areas were regularly stained blue. In the experiments in hypothermia (20° C) the above-mentioned areas were regularly examined, the dura mater in 85 experiments out of 88 animals infused with trpb in hypothermia (20° C), the hypophysis and epiphysis in 79 experiments and the supraoptic crest in 22 experiments. The series with investigation of the areas normally permeable to trpb in chapter IV was not included in these figures.

The choroid plexuses were stained blue in all experiments. In one experiment (C 260) with a very low blood pressure the median eminence, the supraoptic crest and the area postrema were not stained. The epiphysis was regularly stained. The hypophysis was unstained in 2 experiments (C 279 and C 158) out of 56 examined in this respect on injection into the common carotid artery. In both these experiments the length of the action of trpb *in vivo* was less than 30 minutes. The other areas were stained with the exception of the dura mater, which was unstained in one of the 2 experiments.

On injection with ligation of the external carotid artery the hypophysis was stained in 15 experiments, 3 of these faintly, and unstained in 6 experiments, 3 of these forming part of the series with injection of Umbradil 70 %. A lesser degree of staining of the hypophysis did not seem to be related to a lower blood pressure after the injection or to a shorter action of trpb *in vivo*.

The results of the recording of the staining of the dura mater is presented in table 28. On injection into the common carotid artery the staining was recorded in 62 experiments. In one of these the dura mater was not stained and in 12 experiments it was faintly stained. As seen from the experiments with low molecular dextran a faint staining occurred in 2 of the 3 experiments with a short trpb duration. In the other series there did not seem to be any general tendency towards a relation between the length of trpb duration *in vivo* or blood pressure level during the action of trpb and staining of the dura mater.

On injection into the common carotid artery with ligation of the external carotid artery the dura mater was unstained in one experiment and faintly stained in 12 experiments out of a total of 21 experiments. In the series with injection of Umbradil the blood pressure was low during the action of trpb in the experiments with faint staining of the dura mater. The length of trpb duration *in vivo* and degree of staining of the dura mater did not seem to be related.

In 9 of the 13 experiments with a faintly stained or unstained dura mater, the hypophysis was also faintly stained or unstained.

The results of the assessment of the degree of the injury on macroscopic examination in the experiments in hypothermia are surveyed

Table 28. *Staining of dura mater in hypothermia (20° C). The figures denote number of animals*

Experimental series	Not stained	Faintly stained	Patchy staining	Staining most pronounced on side opposite to injection	Bilateral staining
Injection of saline into common carotid artery					
rinsing with saline	0	2	1	3	1
rinsing with formalin	0	2	0	0	1
no rinsing	0	0	0	2	1
with ligation of external carotid artery	1	1	0	2	0
Injection of air in saline with ligation of external carotid artery	0	0	0	1	1
Infusion of noradrenaline in trpb	0	0	0	0	2
Injection of Umbradil into common carotid artery					
50 %. Appl. time 20 sec.	0	1	3	0	1
50 %. Appl. time 15 sec.	0	2	0	1	2
50 % of 38° C. Appl. time 20 sec.	0	1	0	1	3
70 % of 38° C. Appl. time 20 sec.	0	0	1	2	2
50 %. Infusion of noradrenaline	0	1	0	2	2
50 %. Treatment with low mol. dextran 6 %	0	0	1	0	2
50 %. Treatment with low mol. dextran 10 %					
Trpb duration about 10 min.	1	1	0	0	1
Trpb duration 22—30 min.	0	1 ¹	1	2	4
Trpb duration 60 min.	0	0	0	0	5
Trpb duration 105—120 min.	0	0	0	1	4
Injection of Umbradil with ligation of external carotid artery					
50 %. Appl. time 20 sec.	0	1	0	2	2
50 %. Appl. time 40 sec.	0	5	0	0	0
70 %. Appl. time 40—150 sec.	0	5	0	0	0

¹ Common carotid artery ligated bilaterally.

in table 30. Furthermore, a direct comparison of 2 series was made. At a body temperature of 25° C all the 3 series were mutually compared. In hypothermia (20° C) the series were compared with the series with injection of Umbradil 50 % of room temperature into the common carotid artery with an application time of 20 seconds. All the series

Table 29. Hypothermia (20° C). Treatment with low molecular dextran 10 %. Injection of Umbradil 50 % of room temperature into common carotid artery with an application time of 20 seconds. Ranking of the brains in the 4 experimental series with increasing length of trpb duration in a single series with regard to the degree of lesion. Statistical treatment according to the Kruskal-Wallis one-way analysis of variance by ranks.

Experimental series	Trpb duration 10 min	Trpb duration 22—30 min	Trpb duration 60 min	Trpb duration 105—120 min
Number of animals	3	8	5	5
Sum of ranks	19.5	75	66	70.5
Mean of ranks	6.5	9.4	13.2	14.1

$$H_{\text{corrected}} = 4.91$$

$$0.20 > P > 0.10$$

$$\text{Degrees of freedom} = 3$$

treated with low molecular dextran were mutually compared. Furthermore, the series in hypothermia (25° C) with ordinary cooling were compared with the corresponding series at a body temperature of 20° C. The comparison was made twice by the author. The same end result was obtained on both comparisons but it was found that the brains were ranked in a somewhat different order on the 2 occasions. This is most probably due to the fact that the difference in the degree and extent of the injury in the various brains was too small to admit of a consistent ranking. Only on 2 comparisons was an almost significant difference obtained, namely, in hypothermia (20° C) when the series with injection of Umbradil 50 % of room temperature into the common carotid artery with an application time of 20 seconds was compared with the series with an application time of 15 seconds, and with injection of Umbradil 70 % of 38° C with an application time of 20 seconds, respectively. The tendency seen in table 30 for the series at a body temperature of 25° C with ordinary cooling to be more damaged than the corresponding series at 20° C, was not shown to be significant nor almost significant on direct comparison. Ranking of all the series treated with low molecular dextran in one series according to the degree and extent of the lesion showed only a faint tendency for the lesion to increase with increasing length of trpb duration (table 29). This result would be compared with table 30 in which the group with a trpb duration of 105—120 minutes was rated somewhat lower than the group with a trpb duration of 60 minutes.

The Kruskal-Wallis test shows the mean values of the damage in the groups. The weak tendency found with this test is due to the higher proportion of undamaged brains in the groups with shorter trpb duration. More important is the fact that the maximal degree of the injury did not virtually increase with prolonged trpb duration.

No general tendency towards a relation between the length of trpb

duration *in vivo* and the degree of staining of the dura mater on the base of the skull, respectively, was found with the degree of lesion on comparison within the series. In the whole group with injection of Umbradil into the common carotid artery there was no injury to the BBB in the experiments with faint staining of the dura mater. However, on injection with ligation of the external carotid artery staining of the brain occurred even in experiments with faint staining of the dura mater. It does not seem as if the staining of the brain that took place in hypothermia (20° C) on injection of Umbradil was necessarily related to the degree of staining of the dura mater. Within the series there did not seem to be any general tendency towards a relation between blood pressure level during infusion of trpb and degree of lesion.

The macroscopic rating of the degree of injury did not agree with the result of the microscopic investigation in some brains. Two brains (C 157 and C 181) were judged as negative on macroscopic rating but showed parenchymal staining in more than one place on microscopic investigation. This is in part due to the fact that different criteria were used for the assessment of the injury to the BBB on the macroscopic and microscopic examination. Two brains (C 153 and C 220) were judged as (+), i.e. a tendency to confluent staining on macroscopic rating, but showed no parenchymal staining on microscopic investigation. In the 2 last-mentioned experiments the most positive areas on macroscopic examination were found to consist of abundant staining of the vessel wall down to the capillary level. It was also noted that a staining of the vessel wall was not always observed on the macroscopic examination as streaky or dotted staining. In the comparison made above no correction was performed for these discrepancies between macroscopic and microscopic examination.

D. COMPARISON OF SERIES WITH INJECTION OF UMBRADIL IN HYPOTHERMIA AND NORMOTHERMIA

The volume of injected Umbradil and the injection time were of the same magnitude in normothermia and hypothermia in comparable series. Owing to the lower blood pressure in hypothermia the effective injection pressure was as a rule higher in hypothermia as the injection pressure was kept constant.

The drop in blood pressure on removal of the clamps from the jugular veins was almost nil in hypothermia but more pronounced at normal body temperature, sometimes up to 40 mm Hg.

The decided application time was always obtained with Umbradil 50% of 38° C in hypothermia (20° C), by contrast with normal body

Table 30. Comparison of the series with injection of Umbradil in hypothermia with the series with injection of Umbradil 50 % of room temperature into common carotid artery with an application time of 20 seconds at normal body temperature. Statistical treatment according to Fisher's method for 2×2 contingency tables with the alternatives + + + or more and + + or less, as verified by 2 judges on direct comparison of the series.

Umbradil conc. %	Body temp. °C	Appl. time sec	Further experimental characteristics	Degree of injury on sections							P	
				++++	+++	++	+	(+?)	neg			
INJECTION INTO COMMON CAROTID ARTERY												
50	38	20	Prolonged hypothermia with ordinary cooling Prolonged hypothermia with slow cooling Umbradil of 38° C Infusion of noradrenaline Low molecular dextran 6 % Low molecular dextran 10 % Trpb duration about 10 min. Low molecular dextran 10 % Trpb duration 22—30 min. Low molecular dextran 10 % Trpb duration 60 min. Low molecular dextran 10 % Trpb duration 105—120 min. Umbradil of 38° C	4	6	0	0	0	0	0	0	<0.001
50	25	20		0	0	1	6	1	5	1	<0.001	
50	25	20		0	0	0	3	1	1	1	<0.001	
50	25	20		0	0	1 ¹	4	0	1	1	<0.01	
50	20	15		0	0	0	0	0	5	0	<0.001	
50	20	20		0	0	0	0	6	2	0	<0.001	
50	20	20		0	0	0	0	3	2	0	<0.001	
50	20	20		0	0	0	0	2	3	0	<0.001	
50	20	20		0	0	0	1	0	2	0	<0.01	
50	20	20		0	0	0	0	0	3	0	<0.01	
50	20	20	0	0	0	1	2	5	0	<0.001		
50	20	20	0	0	0	1	2	2	0	<0.001		
50	20	20	0	0	0	0	3	2	0	<0.001		
70	20	20	0	0	1	2	1	1	1	<0.001		
INJECTION WITH LIGATION OF EXTERNAL CAROTID ARTERY												
50	20	20	Umbradil of 38° C	0	0	0	0	1	4	0	<0.001	
50	20	40		0	0	0	3	1	1	0	<0.001	
70	20	40—150		1	0	0	2	2	0	0	<0.001	

¹ On direct comparison of the series this brain was found to be as intense as the faintest in the series at normal body temperature.

temperature. At injection into the common carotid artery it occurred both in hypothermia and normothermia that the blood in the pial vessels was not completely expelled in both arteries and veins during the injection.

On injection with ligation of the external carotid artery the expelling of the blood in the pial vessels during the injection was as a rule complete both at normal body temperature and in hypothermia.

The blood pressure increase during injection of Umbradil 50 % of room temperature with an application time of 20 seconds was lower in hypothermia than at normal body temperature. The lowest blood pressure rise in these experiments at normal body temperature was 24 mm Hg and the highest in hypothermia was 18 mm Hg. The blood pressure rise at normal body temperature was on an average 45 mm Hg and in hypothermia less than 10 mm Hg. The highest blood pressure rise in hypothermia was obtained at injection of Umbradil 50 % of 38° C and the lowest in normothermia in the series treated with hexamethonium bromide. Even here the average blood pressure rise was of about the same magnitude. The blood pressure rise during the injection of Umbradil 50 % with an application time of 20 seconds was thus definitely lower in hypothermia (20° C) than at normal body temperature.

At injection with ligation of the external carotid artery the blood pressure rise during the injection was small both in hypothermia and at normal body temperature.

The time for the refilling of the pial vessels with blood after the end of the injection of Umbradil 50 % of room temperature into the common carotid artery at normal body temperature was only recorded in the series with an application time of 5 to 10 seconds. In these series it was shorter than on injection with an application time of 20 seconds in hypothermia (20° C). Umbradil 50 % that was cooled and injected into the common carotid artery with an application time of 20 seconds at normal body temperature was washed away from the pial vessels after the end of the injection with about the same time lag as in hypothermia (20° C).

On injection of Umbradil 50 % into the common carotid artery with ligation of the external carotid artery it was found that with an application time of 20 seconds the time for the expelling of the blood in the pial vessels was longer in hypothermia. However, with an application time of 40 seconds there was no difference.

The present material does not admit of any conclusion with regard to a possible difference in the length of time for refilling of the pial vessels with blood at normal body temperature and in hypothermia even though it may be noticed that occasionally the time lag for washing away the injected solution was rather prolonged in hypothermia.

Table 31. *Hypothermia (20° C). Experiments with infusion of low molecular dextran with a blood pressure compatible with that in the series treated with hexamethonium bromide at normal body temperature.*

Rabbit No	Blood pressure mm Hg			
	At injection	During trpb infusion	After trpb infusion	30 min. after injection
C 152	65	35	35	50
C 157	50	50	40	40
C 167	70	60	70	50
C 150	40	35	50	40
C 136	50	30	45	45

The blood pressure level during the infusion of trpb was as a rule about 30 to 40 mm Hg at normal body temperature. In hypothermia it was often even lower, about 20 mm Hg. However, in 5 animals which were treated with low molecular dextran during the cooling, the blood pressure before the injection, during the trpb infusion, and for the rest of the experiment up to 30 minutes from the beginning of the trpb infusion was of the same magnitude as in 4 of the animals treated with hexamethonium bromide at normal body temperature (table 31). The degree of the lesion in these animals at a body temperature of 20° C was definitely lower than in the hexamethonium treated animals. The difference was significant on the 2 per cent level (Mann-Whitney U test).

Faint staining of the dura mater was uncommon at normal body temperature but occurred in about a third of the experiments in hypothermia (20° C). More intense staining of the dura mater on the side opposite to the injection site occurred in about the same proportion in hypothermia (20° C) and at normal body temperature. It seemed as if a faint staining of the dura mater occurred in a higher proportion in the experiments with ligation of the external carotid artery than in experiments with no such ligation both at normal body temperature and in hypothermia (20° C). Absent or faint staining of the hypophysis occurred at normal body temperature only in the series with ligation of the external carotid artery in 3 experiments. In hypothermia (20° C) absent or faint staining of the hypophysis seemed to occur more often in the experiments with ligation of the external carotid artery.

In table 30 the series in hypothermia are compared with the reference series at normal body temperature i.e. the 10 experiments with injection of Umbradil 50 % of room temperature into the common carotid artery with an application time of 20 seconds. It is seen that the degree of the injury in hypothermia was never assessed to be as intense in the reference series at normal body temperature with the exception

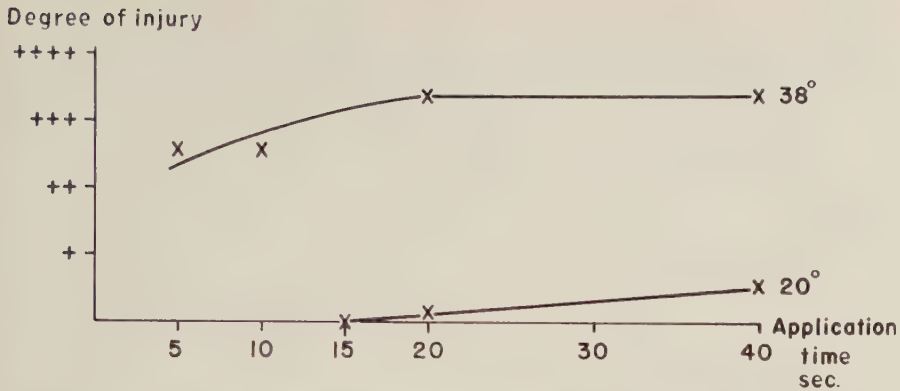


Fig. 6. Relation between degree of injury and length of application time in normothermia (38° C) and hypothermia (20° C). The value of the degree of injury is the mean value for each experimental series (Umbradil 50 %).

of the injury in the animal injected with Umbradil 70 % for 150 seconds. On direct comparison of the series, performed by 2 judges, the same result was obtained with the exception of the brain assessed as ++ in the series denoted prolonged hypothermia with slow cooling in hypothermia (25° C). This brain was found to be damaged to the same degree as the least damaged brain in the reference series at normal body temperature.

In fig. 6 is presented the relationship between the mean values of the degree of the injury and the length of application time for the series with injection of Umbradil 50 % at normal body temperature and in hypothermia (20° C). The series with the shortest application time at normal body temperature was compared directly with the series with the longest application time in hypothermia (table 32) and the difference in the mean values of the degree of lesion was found by 2 judges to be significant. The degree of lesion on injection of Umbradil 70 % of 38° C into the common carotid artery in hypothermia (20° C) was also directly compared with the series with an application time of 5 seconds on injection of Umbradil 50 % of room temperature at normal body temperature. As seen from table 32 the 2 judges showed disagreement in the ranking of adjacent brains in the middle of the rank. However, the tendency was the same in both instances, i.e. the hypothermic series was less damaged than the normothermic one.

The staining of the parenchyma as seen on microscopic investigation was less frequent and less extensive in hypothermia than in the reference series at normal body temperature.

The microscopic picture of the lesion produced by Umbradil 50 %

Table 32. *Direct comparison between the series with the shortest application time at normal body temperature, the series with the longest application time in hypothermia (20° C) and the series with the highest concentration in hypothermia (20° C) on injection of Umbradil. Statistical treatment according to the Mann-Whitney U test. Brace denotes ties.*

Experimental series	Judge	Rank	U	P
Umbradil 50 % of room temperature				
Injection into common carotid artery. Appl. time 5 sec. Body temperature 38° C (A)	I	B B B B A B A A A A	0.5	<0.02
	II	B B B B A B A A A A	1.0	<0.02
Injection with ligation of external carotid artery. Appl. time 40 sec. Body temperature 20° C (B)				
Umbradil 70 % of 38° C				
Injection into common carotid artery. Appl. time 20 sec. Body temperature 20° C (C)	I	C C C C A C A A A A	1.0	<0.02
	II	C C C A A C C A A A	4.0	<0.10

at normal body temperature generally showed large patches of fairly intense confluent staining of the parenchyma, as a rule with abundant staining of the nuclei. In hypothermia the lesion produced with Umbradil 50 % was as a rule confined to the immediate vicinity of the vessels as a blue halo with exceptional staining of parenchymal nuclei. Furthermore stasis was not seen in hypothermia. The vessels in the damaged areas were generally well rinsed in hypothermia by contrast with normothermia. Thus the damaged produced by Umbradil 50 % is considerably reduced in hypothermia, particularly at 20° C.

Even with Umbradil 70 % a marked reduction in the degree and extent of injury occurred at a body temperature of 20° C as compared with the lesion produced with Umbradil 50 % at normal body temperature. A 7-doubling of the application time for Umbradil 70 % in hypothermia (20° C) produced stasis on injection with ligation of the external carotid artery and fairly frequent staining of the parenchymal nuclei. A particular feature on injection of Umbradil 70 % in hypothermia was the consistent occurrence within large areas of a pronounced staining of the endothelial nuclei of the vessel wall with absent or minimal passage of trpb into the brain parenchyma.

DISCUSSION

The cooling time in water in the present material increased with increasing weight of the animals. One factor contributing to the increased

cooling time might be a thicker layer of subcutaneous fat. In man a thick layer of subcutaneous fat was found to decrease the cooling rate (PUGH & EDHOLM, 1955; BAKER & DANIELS, 1956). The temperature of the water did not perceptibly influence the cooling rate unless it was 10°C above the usual one. The infusion of low molecular dextran had no effect on the cooling rate. It might be expected that the sludge preventing effect of low molecular dextran, involving an enhanced capillary circulation, should decrease the cooling time. Shivering was not seen very often. It should, however, be borne in mind that invisible shivering on exposure to cold, detectable only on electromyography, is described by DAVIS & MAYER (1955) as a regular phenomenon. It cannot be excluded that invisible shivering might account for differences in cooling time not attributable to body weight or water temperature. Another factor that might influence the cooling rate is the amount of water used in cooling. This factor was not examined.

The cooling rate appeared to be faster than that reported by BARTLETT (1957) even if the higher body weight of his material is considered. He cooled the animals in Nembutal narcosis with crushed ice. Using an extracorporeal circulation NIELSEN (1954) cooled rabbits to rectal temperatures of about 22°C in about 2 hours.

The pulse and blood pressure at a body temperature of about 20°C after preparation of the animal and before the injection of Umbradil was of the same order of magnitude as that reported for rabbits at the same temperature (NIELSEN, 1954; BARTLETT, 1957).

In the present material the blood pressure and pulse were somewhat higher at a body temperature of 25°C than at 20°C , with the exception of the series that had been kept at a temperature of about 25°C for more than 2 hours after ordinary cooling. The pulse rate and blood pressure of the animals in this series were lower than those in the other series at 25°C and more in agreement with the data at 20°C .

Within the series at a body temperature of 20°C , the animals treated with low molecular dextran during the cooling showed a higher blood pressure and pulse rate than those cooled in the ordinary way to the same body temperature. This may be taken in conjunction with the fact that these animals were often in such a condition after the end of the trpb infusion that it seemed worth while to let them live longer than 30 minutes.

Artificial respiration was necessary in order to keep the animals alive since the doses of Nembutal had to be so large, in order to obtain surgical anaesthesia, that the respiration almost stopped. The cooling procedure was facilitated by the large doses as it was only rarely necessary to give supplementary doses of Nembutal in order to avoid visible shi-

vering. The ventilation was the same as in normothermia, which might be regarded as a hyperventilation of the animals in hypothermia. At a body temperature of 20° C in rabbits cooled with spontaneous respiration, BARTLETT (1957) found a respiration rate that was a sixth of that at normal body temperature. Hyperventilation diminishes the respiratory acidosis encountered in hypothermia on spontaneous ventilation, but a normal pH of the blood could only be obtained by vigorous ventilation (SWAN *et al.*, 1953; OSBORN, 1953). During cooling, BREWIN, GOULD, NASHAT & NEIL (1955) also observed metabolic acidosis, which was enhanced by operative procedures. SEVERINGHAUS, STUFFEL & BRADLEY (1957) found a rise of 0.1 units in pH of blood on surface cooling of dogs to a body temperature of about 25° C with unchanged artificial respiration. They defined normal ventilation in hypothermia as a ventilation in which the carbon dioxide elimination equalled the rate of metabolic production. They found that this normal ventilation was approximated by the maintenance of the same ventilation rate and depth as at normal body temperature. The acid-base equilibrium in the present experiments, however, was not studied. Yet, in view of the discussion above, it does not seem unreasonable to assume that the experimental procedures were such that an extreme low pH of the blood was avoided.

It is known that the temperature gradients in the body of an animal become more pronounced during cooling (BURTON & EDHOLM, 1955; STUFFEL & SEVERINGHAUS, 1956). On surface cooling of dogs to body temperatures of about 26° C GROSSE-BROCKHOFF & SCHOEDEL (1943a) found that the rectal temperature lagged behind the brain temperature up to 10 minutes. COHN & ROSOMOFF (1958) found a difference between oesophageal and brain temperature of up to 6° C during surface cooling and rewarming of cats. COOPER & SELICK (1960) recommend recording from the organ, the temperature of which must be known accurately. This seemed a disadvantage in the present experiments as the needle for temperature recording might cause unpredictable staining of the brain. The brain temperature and the rectal temperature measured 9 cm above the anus were thus studied in a special experimental series since such measurements were only exceptionally performed in the BBB experiments. The temperature of the brain and the rectum measured 9 cm above the anus showed a very good agreement, in contrast to rectal temperatures 5 cm above the anus which showed less good agreement, mostly with differences of about 1—2° C up to 3.5° C (unpublished data).

The amounts of Umbradil injected in series comparable with regard to length of application time and mode of injection were about the same in hypothermia, 25° C and 20° C, and in animals treated with low mole-

cular dextran during cooling. The volumes were also about the same as in comparable experiments in normothermia. It was noticed that in hypothermia (20° C) with an application time of 20 seconds at injection into the common carotid artery, the injected volumes of Umbradil 70 % of 38° C, Umbradil 50 % of room temperature and Umbradil 50 % of 38° C increased in the order given. The viscosity of the solutions decreased in the same order. The amounts of saline in the control experiments were higher than the amounts of Umbradil. That the amounts injected increase with a decrease in viscosity with other conditions being equal is in agreement with Poiseuille's law.

It is particularly noteworthy that at injection into the common carotid artery with ligation of the external carotid artery, the injected amounts were about the same in hypothermia as in normothermia. This observation might seem to be invalidated as an anomalous departure of the occipital artery from the internal carotid artery was not checked. Yet this source of error would play a part only in case the anomaly was only present in all the experiments in hypothermia and in none of the experiments at normal body temperature, which is unlikely.

The time from the beginning of the injection to the filling of the pial vessels with the injected solution was about the same in the various series in hypothermia and showed no difference from normothermia. In the literature, evidence has been presented showing that the cerebral vascular resistance is increased (ROSOMOFF, 1956a; ALBERT & FAZEKAS, 1956; BERING *et al.*, 1956). A reduction of the caliber of the pial vessels up to 50 % in cats in hypothermia (25° C) was reported by MEYER & HUNTER (1957). However, GURDJIAN, THOMAS & WEBSTER (1960) found no change in the pial vessels of monkeys cooled to temperatures down to about 3° C. On selective cooling of the brain through the carotid arteries a drop in the perfusion pressure at temperatures below 30° C was regularly observed (SENNING & OLSSON, 1956; LUND *et al.* 1958; LOURIE *et al.*, 1960). These latter observations seem to be more in accordance with the cold vasodilatation observed in the peripheral arteries (PAPPENHEIMER, EVERSOLE & SOTO-RIVERA, 1948). An increased resistance to intracarotid injections in hypothermia cannot be excluded by the observations in the present experiments. Even though the time from the start of injection to expelling of blood in the pial vessels was virtually unchanged in hypothermia as compared with normothermia, this might be due to the difference in effective injection pressure. However, it was found that about the same volumes of Umbradil 50 % were used on injection into the internal carotid artery also in series with corresponding orders of effective injection pressure. This does not mean that the cerebral vascular resistance is not increased in hypothermia:

The injected solutions might dilate the vessels also at such body temperatures.

It is noteworthy that the decided application time was regularly obtained at injection of Umbradil 50 % of body temperature into the common carotid artery in hypothermia by contrast with normal body temperature. This also applies to the injections of saline. The difference with regard to the length of the obtained application time at normal body temperature with Umbradil 50 % of room and of body temperature was assumed to be due to the difference in viscosity in combination with a possible difference in the reactions of the vessels. The difference in behaviour of Umbradil 50 % of 38° C at normal body temperature and in hypothermia might be due to the difference in effective injection pressure and possibly less pronounced vascular reactions in hypothermia.

The time for the refilling of the vessels seemed to be longer in experiments where the blood pressure was low during the injection. The data in hypothermia are only comparable with those at normal body temperature for the series with injection of Umbradil 50 % with ligation of the external carotid artery and shows no difference for injection with an application time of 40 seconds. The time for refilling of the pial vessels after the end of the injection in the series with an application time of 20 seconds with ligation of the external carotid artery was shorter at normal body temperature than in hypothermia. On injection of saline into the common carotid artery in hypothermia (20° C), the pial vessels seemed to refill with blood in a somewhat shorter time than on injection of Umbradil 50 % into animals treated with low molecular dextran, in spite of the somewhat higher blood pressure in the latter animals.

The blood pressure changes during the injection of Umbradil were slight in hypothermia and as a rule no fall in the blood pressure occurred at the opening of the jugular veins. The rise in the blood pressure was slight but was on an average somewhat higher on injection of Umbradil 50 % and 70 % of body temperature. The increase was definitely lower than that at normal body temperature and as a rule did not exceed the small blood pressure increase in hexamethonium treated animals at normal body temperature. The blood pressure rise during injection of Umbradil into the common carotid artery at normal body temperature was tentatively explained as being in part elicited through the sympathetic nervous system. Some of the reflexes of the vascular system in hypothermia have been investigated, particularly the baroreceptor reflexes of the carotid sinus. GROSSE-BROCKHOFF & SCHOEDEL (1943b) found that the carotid sinus reflex was absent at 26° C in dogs in deep urethan narcosis. BRENDEN, ALBERS & USINGER (1958) were able to

elicit the carotid sinus reflex after bilateral occlusion of the carotid arteries in dogs at body temperatures of 25—20° C if the narcosis was superficial. Preserved though diminished baroreceptor and chemoreceptor reflexes of the carotid sinus in hypothermia have also been reported by NASHAT & NEIL (1955), MALMÉJAC *et al.* (1959) and SALZANO & HALL (1961). The possibility cannot be excluded that the virtual absence of the blood pressure response to Umbradil in hypothermia might in part be due to the deep narcosis in the present experiments.

The assessment of the capillary circulation in the bulbar conjunctiva gave the impression that there was a better circulation in hypothermia (20° C) in animals treated with low molecular dextran than in an untreated group. Thus the capillary circulation was improved by the infusion of low molecular dextran, which is in agreement with the results obtained by GELIN & LÖFSTRÖM (1954), LÖFSTRÖM & ZEDERFELDT (1957b) and LÖFSTRÖM (1959b), who found that infusion of low molecular dextran prevented or diminished intravascular aggregation and preserved a good capillary circulation.

The increase in blood pressure on infusion of noradrenaline was on an average about 50 mm Hg, from about 30 mm Hg to about 80 mm Hg. This is in accordance with the results of SALZANO & HALL (1961), who found a 100 per cent increase in blood pressure on infusion of 2 µg/kg body weight (length of time for infusion is not reported) in dogs at blood temperatures of about 28° C.

The effect of noradrenaline was also elicited, even though less easily when it was solved in trpb and infused in control animals in hypothermia. In the experiments with injection of Umbradil the blood pressure rise was not elicited when noradrenaline was infused in the trpb solution. The reason for this is not clear.

On infusion of trpb in hypothermia, it occurred that the animals succumbed during, or after the end of, the trpb infusion. This occurred irrespectively of the level of hypothermia, extent of preparation, mode of injection, and type of injected solution. The infusion of 100 ml of a solution intravenously is certainly a large amount in a rabbit and might exert a heavy strain on the heart with dilatation, particularly on rapid infusion. However, no relation between the rate of infusion and length of survival was found.

In animals with infusion of trpb only at normal body temperature, the blood pressure rose during the first part of the infusion and then generally fell slightly. In the experiments with intracarotid injection at normal body temperature the same thing happened, occasionally with a rise in blood pressure after the end of the trpb infusion. The blood pressure was as a rule not restored to the pre-injection level.

An initial rise of the blood pressure often occurred during the infusion of trpb in hypothermia. Then a decrease in pulse amplitude (as recorded on the mercury manometer) mostly took place with a slowing down of the heart rate and a decrease of blood pressure, which fell to zero levels in the animals that succumbed. It seems as if the drop in blood pressure occurring in animals at normal body temperature was particularly marked in these animals. In guinea pigs at normal body temperature, BROMAN (1941b) observed a fall in blood pressure which was more pronounced than in rabbits and cats.

During the infusion of trpb in animals treated with low molecular dextran the blood pressure was often unimpaired. It is possible that the favourable effect of low molecular dextran on the capillary circulation makes the heart more fit to withstand the strain of the infusion of the trpb solution.

As seen in chapter IV the areas normally permeable to trpb were stained in hypothermia. This was mainly confirmed in the control series and in the BBB experiments. The dura mater, however, seems to be an exception in the sense that it was not regularly stained. To judge from the series in hypothermia in chapter IV and from the series with infusion of low molecular dextran, the staining of the dura mater was more prone to be normal when the action of the trpb was long. However, this was not the general trend in the other series with injection into the common carotid artery.

In the series with injection into the common carotid artery with ligation of the external carotid artery the proportion of faint staining of the dura mater and the hypophysis was higher both in hypothermia and normothermia than on injection into the common carotid artery. The reason for this is not clear. It did not seem to be related to the staining of the brain, which was fairly pronounced in the pia mater and the outer layer of the brain in the series with injection of Umbradil 70 % even in experiments with faint staining of the dura mater in hypothermia. At normal body temperature the staining of the brain did not seem to be related to the staining of the dura mater when the other areas normally permeable to trpb were stained.

The question arises whether it is possible to demonstrate an injury to the BBB with trpb in hypothermia. The author's arguments for this possibility are the following: The areas normally permeable to trpb in the brain were stained to the same degree in normothermia and hypothermia (chapter IV); the needle track for temperature recording in the brain was stained; in some experiments injection of Umbradil 70% gave staining of the brain parenchyma with staining of the parenchymal nuclei and in some experiments uniform staining of the parenchyma in

certain parts of the brain. On injection of Umbradil 50 % in hypothermia (25° C) one exceptional brain showed the same degree of injury as in normothermic animals.

Furthermore, JEPSSON (unpublished data) found that injection of sodium desoxycholate in a 0.05 per cent solution in saline into the common carotid artery in rabbits with an application time of 20 seconds gave the same degree of lesion in the normothermic animals as in those with a body temperature of 20° C on infusion of trpb. It seemed, however, as if the degree of the injury produced by sodium desoxycholate in hypothermia was dependent on the length of the trpb duration. It occurred that the lesion in hypothermia was less pronounced than at normal body temperature when the length of the trpb duration was the same as that at normal body temperature, i.e. 15 minutes, but showed the same degree when the length of trpb duration in hypothermia was increased to 30 minutes (plate IV). The sodium desoxycholate might act in a different way than Umbradil on membranes as it is surface-active (THORÉN, 1958).

LOURIE, WEINSTEIN & O'LEARY (1960) claimed that injection of concentrated roentgen contrast media in cats at brain temperatures of 20° C showed staining of the brain, which appeared on injection of comparatively small amounts of trpb. It is not known whether these lesions were compared with normothermic lesions.

Thus the available evidence shows that trpb is capable of indicating damage to the BBB in hypothermia.

In some of the experiments the dura mater was only faintly stained. The question arises whether this means that the amount of trpb that has reached the brain has not been enough to show an injury to the BBB. No comparison of the staining of the dura mater and of the brain in cases of damage to the BBB has been reported. BROMAN (1949) states that a distinct blue staining of the dura mater and the choroid plexuses is a criterion that a sufficient amount of the trpb solution has been given.

In hypothermia it was found that with the injection of Umbradil 70 % a relatively pronounced staining in some parts of the brain was obtained in spite of a faint staining of the dura mater. Furthermore, on injection of Umbradil 50 % in hypothermia the lesion of the brain was still faint even in experiments with a normal staining of the dura mater.

It does not seem likely that the explanation of the faint staining produced by Umbradil 50 % with an application time of 20 seconds in hypothermia was that the trpb had not time enough to take effect, since the staining was still faint with survival times up to 2 hours, and the mean values of the injury showed but a weak tendency to increase and the maximal lesion did not increase at all.

It does not seem likely that this faint staining was due to smaller

amounts of the damaging agent passing through the brain, since about the same volumes were used in hypothermia and normothermia also in the experiments with ligation of the external carotid artery.

It may be possible that the intracarotidally injected agent did not pass through the brain capillaries but passed down into the subclavian arteries owing to the high effective injection pressure. This is less likely to happen on injection into the common carotid artery. JEPSSON & OLIN (1960) found filling only to the anterior part of the basilar artery on injection into the common carotid artery with an effective injection pressure of about 250 mm Hg in the normothermic rabbit. Furthermore, in the experiments with infusion of noradrenaline the effective injection pressure was of about the same magnitude as that in normothermic animals, and in some of the animals with infusion of low molecular dextran the blood pressure was about the same as that in normothermic animals treated with hexamethonium bromide and hence with an effective injection pressure of the same magnitude. An injury to the BBB was found in the normothermic animals, but not in the hypothermic ones.

Furthermore, on injection of Umbradil 70 % into the common carotid artery and with ligation of the external carotid artery, staining of the vessel wall down to the capillary levels was seen. This also supports the assumption that intravascular aggregation in the vessels of the brain in hypothermia is no absolute obstacle to intracarotid injections and intravenous infusions. Moreover, the contrast medium cannot pass by the capillaries as there are no arterio-venous anastomoses in the mammalian brain (WENTSLER, 1936; CAMPBELL, 1938; SCHARRER, 1940; STEEGMAN & DE LA FUENTE, 1959).

In the present experiments the effective injection pressure was about 180 mm Hg when the injections were made with ligation of the external carotid artery. When the injections were made in this way in the series with an application time of 40 seconds, it was found that the cerebellum was not damaged. This shows that the contrast medium in the basilar artery did not reach far enough to fill the superior cerebellar artery in damaging amounts and concentrations. On injection of contrast medium into the internal carotid artery, JEPSSON & OLIN (1960) found that an effective injection pressure of about 100 mm Hg filled the upper part of the vertebral arteries and that the contrast medium was still within the vertebral arteries with an effective injection pressure of about 150 mm Hg. It is possible that on injection with ligation of the external carotid artery, part of the injected contrast medium may pass through the anastomosis between the internal and the external ophthalmic artery. JEPSSON & OLIN (1960) found that this passage took place at an effective injection pressure of about 100 mm Hg.

In the present experiments the effective injection pressure was above 100 mm Hg, but higher in hypothermia than at normal body temperature. This need not necessarily mean that substantially more contrast medium passes over into the external carotid artery with an increase of the effective pressure, as the anastomosis between the external and the internal carotid artery has as a rule a smaller diameter than the vertebral arteries (JEPPSSON & OLIN, 1960).

The experiments with infusion of low molecular dextran showed that the decrease in the injury to the BBB in hypothermia was not caused by the intravascular aggregation occurring in hypothermia, since diminishing of the intravascular aggregation did not increase the injury to the BBB.

As shown by the experiments with hexamethonium bromide at normal body temperature and the experiments with noradrenaline in hypothermia, the level of the blood pressure at the time of the injection into the carotid artery had no influence upon the degree of the lesion.

The factors that cause the trpb to pass into the brain parenchyma when the BBB has been injured are not known, any more than the influence of decreased temperature *per se* or the changes that occur in hypothermic animals. It may be assumed that in the case of a damage to the BBB the same factors are at work as in capillaries in general, so the following reasoning might be applied: Certainly a concentration gradient exists as trpb is not normally found in the brain parenchyma. As trpb seems to be bound to the cells it is possible that this concentration gradient might be reduced during the action of the trpb. After the passage through the capillary wall and possibly the glial membrane, the trpb might still be transported in a water phase even if the extracellular space is small (100—200 Å) according to electron microscopic investigations (SCHULTZ, MAYNARD & PEASE, 1957; LUSE, 1960a, b; HAGER, 1961.)

A decrease in temperature most likely reduces the diffusion rate of the trpb. This reduction would be fairly small if the diffusion process is a "physical" one but greater if a "chemical" process is involved (for references see BAYLISS, 1959). In an investigation on the frog skin (GELLHORN & GELLHORN, 1929) found that the rate of the passage of acid and basic dyestuffs was reduced during a fall of temperature with a factor of about 2 for an interval of 10° C. The factor was the same for basic dyestuffs tested on muscle membranes, but slightly above 1 for acid dyes. This makes it probable that the penetration of basic dyes is due to chemical processes. In a perfused hindleg preparation in the cat PAPPENHEIMER (1953) found a decrease in the filtration coefficient (expressing the proportionality between flow and pressure across the capillary wall) from 35° C to 10° C, which was of the same order of magnitude as the change in viscosity of water.

In hypothermia the blood flow is slower than at normal body temperature (BRENDDEL, 1957). A decrease in blood flow diminishes the amount of a substance passing across the capillary wall per time unit (RENKIN, 1959).

These factors have not been studied separately and quantitatively in normothermia and hypothermia and the only way to compensate for them in hypothermia is to increase the length of the action of trpb. As mentioned above, it was empirically found in the experiments with sodium desoxycholate that it was possible in hypothermia to obtain as intense a staining as in normothermia by doubling the trpb duration. Furthermore, the staining of the areas of the brain normally permeable to trpb was not virtually increased by an increase in the trpb duration. In the experiments with Umbradil the time for the action of trpb was increased 8 times without any virtual increase in the degree of the staining. It is thus unlikely that the decreased injury to the BBB in hypothermia produced by Umbradil is due to the slower flow of the indicator agent or to a slower diffusion rate in hypothermia.

The importance of the filtration pressure for the passage of trpb into the brain parenchyma in the case of an injury to the BBB is not known. Those experiments in hypothermia in which the blood pressure during the infusion of trpb was the same as that at normal body temperature, showed that the reduced staining in hypothermia in the Umbradil experiments could not be explained by the low blood pressure during the infusion of trpb.

The effect of hypothermia on the binding of trpb to plasma albumin has not been investigated. However, it is unlikely that such changes are of importance in the present experimental conditions as it has been demonstrated that temperature changes and pH changes from 6 to 9 only slightly effect the binding of ions by serum albumin (see KLOTZ, 1953). Furthermore, there is evidence to the effect that the damage to the BBB produced by contrast media makes possible the entrance of albumin into the brain parenchyma. EDSTRÖM (1961) found passage of radioactive iodinated human serum albumin after intracarotid injection of Triurol. This is most likely valid for iodopyracet compounds too, since BASSETT *et al.* (1953) found passage of Evans blue into the brain parenchyma after intracarotid injection of Diodrast. Evans blue is firmly bound to the plasma albumin and behaves like radioactive iodinated albumin on injection into the blood (SCHULTZ *et al.*, 1953).

At a body temperature of 25° C it was found that prolongation of the cooling time did not perceptibly change the degree of lesion. It was, however, noticed that on microscopic examination the lesion gave the impression of being less pronounced in the series with cooling prolonged

by keeping the animal at a body temperature of about 25° C for about 2 hours after initial normal cooling. In this series the blood pressure and the pulse rate were also lower than in the other 2 series in hypothermia 25° C. It could not be excluded that the fairly small difference between this series and the other two might be due to the above-mentioned difference in the circulatory status. The results obtained at a body temperature of 20° C, however, do not support this suggestion.

The macroscopic picture of the injury to the BBB in hypothermia on injection of Umbradil 50 % was characterized by streakiness, dottiness and patchiness, in contrast to the appearance of the lesion at normal body temperatures, which as a rule was uniform but could be patchy in areas with a probably low concentration of the contrast medium or a short application time. In hypothermia the vessels were generally well rinsed in the damaged areas by contrast with the case at normal body temperature.

The first question that arises is whether these lesions in hypothermia are due to artefacts. However, no such lesions were obtained in the control experiments (chapter III). In the experiments with no rinsing of the vessels *post mortem*, a small extravasation of trpb was seen on rare occasions in hypothermia. The frequency of dots was considerably greater than in the experiments with injection of Umbradil and moreover the vessels were well rinsed. It is improbable that the lesion is caused by air emboli as air in the injection system was carefully avoided. Furthermore, the lesion produced by Umbradil 50 % had a different appearance than that caused by air in the injection system in contrast to the damage caused by Umbradil 70 %. It is clear that the damage in the Umbradil experiments in hypothermia is due to the injection of the Umbradil. Since the degree of the lesion in hypothermia increased with the length of the application time as in the experiments at normal body temperature, it seems most likely that the dotty and streaky appearance of the lesion in hypothermia is due to a local increase in the application time or possibly to a variation in the time for refilling of the vessels with blood.

The possibility that the lesion was due to an intravascular precipitation of Umbradil 50 % does not seem probable as Umbradil 50 % of room temperature is not prone to precipitate.

On the injection of Umbradil 70 % of 38° C in hypothermia the lesion was characterized by pronounced staining of the pia mater and of the outer layer of the cortex, while otherwise there was only a faint staining, or none at all, of the brain parenchyma. On the microscopic investigation it was found that the vessel wall was markedly stained and that there was staining of the endothelial nuclei in most parts of

the brain, even in those without any passage of trpb into the brain parenchyma.

As Umbradil 70 % of room temperature easily precipitates when the needle of the syringe is put down into the ampoules, it was necessary to use Umbradil of 38° C even in the experiments in hypothermia. This implies that when the contrast medium reaches the vessel wall the temperature at that site is higher than 20° C. It also means that the temperature of the Umbradil solution is less than 38° C. The results obtained with Umbradil 70 % in hypothermia might not be representative of the effect of the contrast medium at the actual body temperature. However, when Umbradil 50 % of 38° C was used in hypothermia (20° C) it was found that the temperature of the contrast medium did not influence the character of the lesion. The decrease of the temperature of the contrast medium during the injection might possibly cause precipitation of the contrast medium and the changes seen on injection of Umbradil 70 % may thus be caused by solid emboli. On the other hand this does not seem likely as the lesion produced by solid emboli is not made visible by the trpb until 1—3 hours after the injection (BROMAN, 1940a). The interpretation of the results with regard to the location of the BBB will be discussed in chapter VII.

SUMMARY

The injury to the BBB produced by Umbradil 50 % was markedly reduced in hypothermia. The reduction had a tendency to be more pronounced at a body temperature of 20° C than at one of 25° C. The reduction of the lesion persisted also when the trpb solution was permitted to act for up to 2 hours. Infusion of low molecular dextran during cooling did not restore the lesion to normothermic levels. The reduction of the lesion was not due to the low blood pressure in hypothermia. The lesion produced by Umbradil 70 % in hypothermia 20° C was also reduced on comparison with the lesion produced by Umbradil 50 % at normal body temperature. Unexpected location of the lesion could be found in hypothermia.

**THE MODE OF ACTION OF UMBRADIL IN PROVOKING
INJURY TO BLOOD-BRAIN BARRIER. THE ANATOMIC
LOCUS FOR THE RESTRICTION OF PASSAGE OF
TRYPAN BLUE INTO BRAIN PARENCHYMA.
A DISCUSSION**

The mode in which Umbradil acts on structures restricting the passage of trpb from blood into brain parenchyma is not clear. BROMAN & OLSSON (1949) and BASSETT *et al.* (1953) found that the damaging effect of the contrast medium could not be explained by its hypertonicity only. In a study on the degree of injury to the BBB produced by different contrast media, HOPPE (1959) found no relation between the iodine content of the contrast media and their damaging effect. The lack of relation between the hypertonicity and the iodine content of contrast media, respectively, and their damaging effect has proved to be valid for the kidney too (BERG, IDBOHRN & WENDEBERG, 1958). In an investigation of the damage produced by iodopyracet and glucose to the endothelium of the facial vein in the rabbit, ZINNER & GOTTLÖB (1959a) found that the effect of the contrast medium could not be explained by the osmotic effect only. Using the same technique, ZINNER & GOTTLÖB (1959b) compared the effect of iodopyracet, sodium acetrizate and the diatrizate compound, Urografin, and found that the slightest lesion to the endothelium was caused by the latter substance, which has a considerably higher viscosity. In a similar investigation of the endothelium of the lateral vein on the tail of the rat, MERSEREAU & ROBERTSON (1961) found no difference in the damaging effect of Diodrast 70 % and Renografin 76 %. Urokon 70 % generally produced a severer endothelial damage.

HOPPE (1959) found it conceivable that the injury to the BBB produced by contrast media may be initiated by their hypertonicity, while their "specific local tissue toxicity inherent in the chemical structure of the individual compound may complete the breakdown of the blood-brain barrier."

The observation by WINZER, LANGECKER & JUNKMANN (1954) that iodopyracet diminishes tissue respiration indicates that these compounds may act as enzyme inhibitors.

The vasodilatation evoked by the contrast media has by BLOOR,

WRENN & MARGOLIS (1951) and MARGOLIS *et al.* (1959) been considered to be the cause of their impairment of the BBB.

It was shown in chapter VI that the decrease of the lesion produced by Umbradil in hypothermia could not reasonably be explained as a methodological error caused by the hypothermic state of the animal. It is not clear whether the profound changes produced in the physiology of the mammalian body under hypothermia may interfere with the damaging action of the contrast media. It is known, however, that the temperature *per se* influences the velocity of reactions, which decreases with decreasing temperature. This relation is known as the Arrhenius equation, according to which the logarithm of the velocity of the reaction is proportional to the reciprocal of the absolute temperature (for references see JOHNSON, EYRING & POLISSAR, 1954; PRECHT, CHRISTOPHERSEN & HENSEL, 1955; BAYLISS, 1959). The relatively great decrease in the degree of injury to the BBB produced by Umbradil obtained over a temperature interval of about 20° C, might be an expression of the possibility that a chemical process is involved.

It was found that the vessel wall could be damaged by the contrast medium in hypothermia without any observable passage of trpb into the brain parenchyma (chapter VI). This might conceivably imply that 2 factors with different temperature coefficients are at work in the production of the injury to the BBB by Umbradil. According to the hypothesis put forward by HOPPE (1959), contrast media may be injurious to the BBB by a combination of hypertonicity and a chemical factor bound to the structure of the contrast media. This hypothesis would fit in with the results of the present investigation as it is likely that the effect of hypertonicity is less influenced by temperature than the effect of the chemical factor. It must, however, be borne in mind that, even though damage restricted to the vessel wall occurred in the experiments with Umbradil 50 %, this damage was as a rule confined to larger vessels. Furthermore, entirely undamaged areas were found in these experiments, which may indicate that the damage to the vessel wall is not wholly independent of the temperature. These differences between different areas in the same brain may be an expression of a difference in length of application time or in amount of contrast medium passing through a vascular region. The experiments with Umbradil 70 % in hypothermia showed that if a lowered temperature diminishes the damage to the vessel wall, it is easily counteracted by an increase in the concentration.

The fact that exceptionally a pronounced injury to the BBB could occur in the latter experiments may also be an expression of a difference in length of application time or in amount of contrast medium passing through a vascular region.

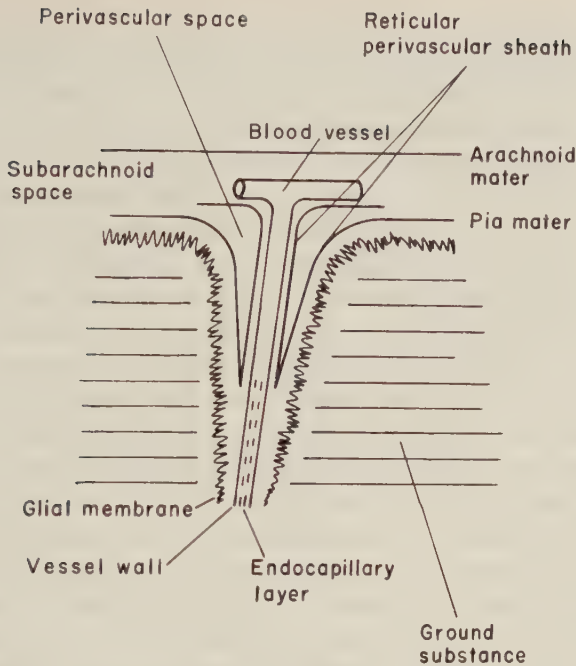


Fig. 7. Schematic representation of conceivable structures intervening between blood and neuron. Based mainly on the paper by BRIERLEY (1957).

The effect of the hypertonicity of the contrast media on the vessel wall might be further elucidated by studies with diatrizoate compounds which seems to be less injurious to the BBB (see review in chapter V). However, no study with particular reference to the staining of the vessel wall has been reported. In this connection it should be pointed out that MERSEREAU & ROBERTSON (1961) showed that the endothelium of large veins can be damaged by diatrizoate compounds also.

It is not clear whether the vasodilating effect of Umbradil on the cerebral vessels is reduced in hypothermia. However, it is known that CO_2 dilates the cerebral vessels at a body temperature of 25°C (KLEINERMAN, 1956). Thus it seems as if the cerebral vessels might still be capable of dilatation in hypothermia.

It is probable that the injurious action of Umbradil on the BBB is due to the chemical structure of the substance.

The use of trpb in BBB studies with concomitant microscopic investigation invites a discussion of the anatomic locus of the BBB. The conceivable structures that may be the morphological substrate of the BBB have recently been reviewed by WOOLLAM & MILLEN (1954) and BRIERLEY

(1957). A schematic drawing of these structures is presented in fig. 7. The endocapillary layer is a hypothetical protein layer which has been assumed to exist on the basis of experiments with perfusions of vessels in the frog. If the perfusate did not contain proteins, edema or extravasation of dye occurred (for references see BRIERLEY, 1957).

The capillary wall has for a long time been considered an obstacle to the passage of trpb from blood to brain parenchyma. LEWANDOWSKY (1900) thought that the capillary wall in various organs might have different permeability qualities. SPATZ (1934) found that the capillary wall was not stained in the normal brain. Further arguments for locating the BBB in the capillary wall were obtained in studies concerned with the abnormal passage of trpb into the brain parenchyma, which showed that the vessel wall was stained (BROMAN, 1940a, 1949; GRÖNTOFT, 1954). Similar conclusions were reached by AIRD & STRAIT (1944) in studies with trypan red and by RODRIGUEZ-PERALTA (1957) in studies with aminoacridine. BROMAN & LINDBERG-BROMAN (1945) found that a greater concentration of e.g. bile salts was necessary to produce extravasation of trpb when the bile salt was applied on the outside of the pial vessels than on intracarotid administration. These findings were adduced by them as further evidence that the BBB is located in the capillary wall.

According to KROGH (1946), the selective passage of substances from blood to brain could be explained by the assumption that the capillary wall of the cerebral vessels has special properties. He pointed out the similarity between the permeability of these vessels and cell membranes in general. CRONE (1961) determined the permeability coefficients for various substances passing from blood to brain and found that these coefficients ranked in the same order as that valid for cell membranes in general. Also DAVSON & SPAZIANI (1959) and DAVSON, KLEEMAN & LEVIN (1961) located the factor restricting the passage of substances from blood to brain in the capillary wall. This hypothesis was based on their experimental evidence of the existence of an extracellular space in the brain.

The theories reviewed above have led to a search for special morphological properties of the capillary wall in the brain which form a contrast to those of the capillary wall in other regions of the body. In these regions the wall permits the passage of trpb and other substances which only selectively pass into the brain parenchyma. This search has in recent years been concentrated to studies by electron microscopy. FARQUHAR & HARTMANN (1956) and MAYNARD, SCHULTZ & PEASE (1957) found that the capillary endothelium in the brain of the rat formed a continuous layer which according to the latter authors lacked fenestrations as seen in the

kidney. In their electron microscopic studies in various animals and organs BENNET, LUFT & CHAMPTON (1959) found that the brain capillaries might differ from the muscle capillaries in that a possibly complete perivascular cellular investment is present. HAGER (1961) found that the endothelium of the brain capillaries in the golden hamster did not differ from that of capillaries in most other parts of the body. He did not describe fenestrations in the brain capillaries. NIESSING & ROLL-HÄUSER (1954) studied the structure of the brain vessels in man with polarization microscope and found that the endothelium consisted of protein layers with scattered insules of lipoids. They expressed the opinion that the factor restricting the passage of substances from blood to brain was located in the capillary wall. CRONE (1961) found that the muscle capillaries had a larger permeability to inulin and saccharose than might be expected from the electron microscopic appearance of these capillaries.

The present investigation confirms the assumption that the capillary wall is an obstacle to the passage of trpb into the brain parenchyma. In the normal brain no staining of the parenchyma was seen either in normothermia or in hypothermia. On infusion of trpb with no rinsing of the vessels, no positive evidence of regular staining of the vessel wall was obtained. It must, however, be borne in mind that it is difficult to decide from the microscopic picture whether the capillary wall is stained or not when no rinsing is performed. It might be expected, however, that if the capillary wall was stained, a higher intensity would be found in the wall, as seen in the experiments with damage to the BBB in unrinsed areas. This was not found in the unrinsed normal brains and it is thus reasonable to assume that the capillary wall was not stained in these experiments. It is clear, however, that no staining of the endothelial nuclei existed in the normal, unrinsed brain. In the rinsed areas of the damaged brains the vessel wall was definitely stained, in clear contrast to the control experiments. The contrast medium certainly damages the vessel wall. In the experiments with Umbradil 70 % a pronounced damage to the vessel wall with none or only a faint staining of the brain parenchyma was a regular feature. This finding makes it necessary to discuss the question whether structures outside the vessel wall, together with the capillary wall, might be responsible for restricting the passage of trpb into the brain parenchyma.

The possible structures are the reticular perivascular sheath, the ground substance and the glial membrane.

For the historical aspects on the perivascular space the reader is referred to reviews in the works by SCHALTENBRAND & BAILEY (1928) and WOOLLAM & MILLEN (1954). In recent years several reports have agreed that the perivascular sheath does not reach beyond the capillary level

(PATEK, 1944; MILLEN & WOOLLAM, 1954; DEMPSEY & WISLOCKI, 1955; BAIRATI, 1958; HAGER, 1961). In the rabbit the perivascular space seems to be found around vessels about 20 μ in diameter (PATEK, 1944). In man, BAIRATI (1958) reported that the perivascular space was hardly visible around vessels smaller than 30—50 μ . In the present investigation vessels of 10 μ or less have been regarded as capillaries. Staining was occasionally found around vessels about 30 μ in diameter. With regard to the variations of the measurements of the width of brain vessels (for references see BRIERLEY, 1957) it is not clear whether the dye in this case has passed through the perivascular space or not.

It has been assumed that there is a substance between nerve-cells and neuroglia. According to HESS (1953), this substance, called the ground substance, is stained by the periodic acid-Schiff (PAS) procedure. It was shown that the ground substance identified in this way disappeared around stab wounds inflicted in the brain (HESS, 1955). MILLEN & HESS (1958) suggested that similar experiments with preceding injection of trpb in small doses tend to show that the ground substance is a possible obstacle to the passage of trpb into the brain parenchyma. The existence of a ground substance has been denied by DEMPSEY & LUSE (1958) and HAGER (1961), since electron microscopic investigations have shown that there is no adequate space for a ground substance. Furthermore, it was found by LUMSDEN (1957, 1958) that the astrocytes in tissue cultures were stained by the PAS procedure. This led him to believe that the ground substance was nothing more than glial cytoplasm. Owing to the above-mentioned observations, the ground substance seems to be less probable as a locus for the restriction of the passage of trpb into the brain parenchyma.

According to GÄRTNER (1927) the glial membrane outside the capillary wall is the barrier between blood and brain. The evidence was obtained by long-term experiments with trpb in rabbits whose brains were damaged by stab wounds. The glia, which was concomitantly studied with a special staining technique, was absent in the vicinity of the wound. GÄRTNER also found that the glial membrane was lacking in the neurohypophysis, which is stained by trpb. This fact was regarded as further evidence of the importance of the glial membrane as an obstacle to the passage of trpb into the brain parenchyma.

SCHALTENBRAND & BAILEY (1928) found that the pia mater fused with the glial membrane. This structure was called the pia-glia membrane. They also found that the perivascular space did not exist on the capillary level. SCHALTENBRAND & BAILEY performed an experiment in the cat with simultaneous injection of distilled water into the carotid artery on one side and 10 per cent saline on the other. Care was taken to

keep the same injection rate on both sides. They found dilated perivascular spaces on that side which had been perfused with the hypertonic solution, and collapsed perivascular spaces on the side injected with distilled water. These results were taken to show that the pia-glia membrane is a barrier to the transport of substances from blood to brain parenchyma. TSCHIRGI (1950, 1952) claimed that trpb does stain the vessel wall but stops at the glial membrane in unrinsed brains. It is not clear how TSCHIRGI could obtain positive evidence of staining of the capillary wall in the unrinsed brain. In a recent paper, however, TSCHIRGI (1960) for other reasons withdraws the hypothesis based on these investigations after the criticism of GRÖNTOFT (1954), BAKAY (1956) and RODRIGUES-PERALTA (1957), and seems to be of the opinion that the capillary wall rather than the glial membrane is likely to be the barrier.

The opinion that the glial membrane is the locus for the restriction of the passage of substances from blood to brain has gained some support from recent electron microscopic studies, since this membrane has been found to invest on the capillary wall. However, the results obtained with regard to the completeness of the glial membrane are not unanimous. MAYNARD, SCHULTZ & PEASE (1957) considered the sheath of the end feet of astrocytes to be approximately 85 per cent complete. DEMPSEY & WISLOCKI (1955), VAN BREEMEN & CLEMENTE (1955) and LUSE (1960a) seem to regard the glial membrane as complete. DEMPSEY & WISLOCKI (1955) however, pointed out that these investigations did not show whether it is the ground substance or the glial membrane that acts as a barrier between blood and brain. BENNET, LUFT & HAMPTON (1959) raised certain objections to the technique in the above-mentioned investigations but seemed to accept the completeness of the pericapillary investments as a possible basis for the classification of capillaries.

Of the structures intervening between the capillary wall and the neuron, the glial membrane seems to be the most likely one to explain the discrepancy seen in the experiments with Umbradil 70 % between the severely damaged capillary wall and the slight or absent passage of trpb into the brain parenchyma. It may be questioned whether the non-complete obstacle indicates that this membrane has not been sufficiently damaged or perhaps that the glial membrane is incomplete. In some of these experiments it was also noted that the pia mater, but not the brain parenchyma, was intensely stained. It is possible that this is due to a damage to the vessels before entering the brain, which might result in extravasation of trpb after intracarotid injection of emboli (BROMAN, 1940a) or after application of e.g. bile salts on the outer surface of these vessels (BROMAN & LINDBERG-BROMAN, 1945). The observed discrepancy between the degree of staining of the pia mater and the

brain seems to be a further support of the assumption that the glial membrane might be an additional obstacle to the passage of trpb.

In recent years the discussion of the selective passage of substances from blood to brain has centred on the part played by the extracellular space in the brain. The size of this compartment differs according to the method used for the determination.

ALLEN (1955) found the extracellular space in the rat cerebrum determined *in vitro* with inulin and ferrocyanoide to be about 15 per cent. DAVSON & SPAZIANI (1959) concluded from *in vivo* and *in vitro* studies on the rabbit's brain with I^{131} , sucrose and p-aminohippurate, that the extracellular space ranged from 14 to 22 per cent. DAVSON, KLEEMAN & LEVIN (1961), using sucrose and p-aminohippurate, found the extracellular space in the spinal cord of the rabbit to be about 12 per cent.

Electron microscopic investigations have shown that the space which separates two adjacent cells is of the order 100—200 Å (SCHULTZ, MAYNARD & PEASE, 1957; LUSE 1960a, b; HAGER, 1961). On the basis of an electron microscopic investigation of the brain of a shark (*Scylliorhinus*), HORSTMANN & MEVES (1959) calculated the extracellular space to be about 5 per cent of the total volume. The above-mentioned authors were of the opinion that the restricted passage of substances from blood to brain could be explained by the virtual absence of extracellular space and did not think it necessary to assign special permeability properties to the capillary wall.

If it is assumed that the small extracellular space is the reason why trpb does not pass into the brain it follows that a passage of trpb into the brain parenchyma means that this space has increased. As seen from the discussion of the mode of action of Umbradil this substance is likely to injure the vessel wall and perhaps the glial membrane owing to its chemical structure. Even if Umbradil elicits the passage of trpb by means of a vasodilatation, this process would only mean that other compartments of the brain diminish as the brain is enclosed in a box of fixed volume. However, it is possible to assume that the osmotic pressure of Umbradil causes a shrinkage of the brain with an increase of the extracellular space. But it must be borne in mind that it has not been shown that the effect of Umbradil can be attributed to its osmotic pressure only. Furthermore, it is less likely that the effect of the osmotic pressure would be much interfered with over a temperature interval of 20° C.

Thus the fact that Umbradil elicits passage of trpb into the brain parenchyma hardly supports the hypothesis that a non-passage of trpb into the brain parenchyma is due to a possibly small extracellular space.

SUMMARY

The decrease of the injury to the BBB in hypothermia produced by Umbradil is tentatively explained as being due to a decrease in a reaction involving the contrast medium and the structures comprising the BBB.

The investigation supports the hypothesis that the capillary wall is the structure mainly responsible for restricting the passage of trpb into the brain parenchyma. It was found that a pronounced staining of the vessel wall did not necessarily mean that the brain parenchyma was markedly stained. This observation may imply that the glial membrane acts as a supplement to the capillary wall in restricting the passage of trpb. The present investigation does not seem to support the hypothesis that a small extracellular space in the brain explains the fact that trpb does not normally pass into the brain parenchyma.

GENERAL SUMMARY

This investigation is concerned with the effect of intracarotid injection of iodopyracet (Umbradil) on the passage of trypan blue (trpb) into the brain parenchyma of the rabbit in normothermia and in hypothermia.

Passage of trpb into the brain parenchyma is denoted as injury to the blood-brain barrier (BBB).

1. It was found that the injury to the BBB produced by Umbradil was markedly reduced in hypothermia in the range of 25°C to 20°C .

2. It was found that the decrease in the injury could not be explained by interference of the hypothermic state of the animal with the flow of the contrast medium through the vessels of the brain in hypothermia.

3. It was found that the decrease in the injury could not be explained by interference of the hypothermic state of the animal with the passage of trpb into the brain parenchyma in case of damage to the BBB.

4. The decrease of the injury is tentatively explained as an effect of the hypothermic state of the animal on a reaction between Umbradil and the structures which restrict the passage of trpb into the brain parenchyma.

5. The investigation supports the hypothesis that the restriction of the passage of trpb into the brain parenchyma is located in the capillary wall. It is, however, not unlikely that the glial membrane may be a supplement to the capillary wall in restricting the passage of trpb into the brain parenchyma.

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TABLES

Special abbreviations used in the following tables.

Blood expelled in pial arteries and veins (complete expelling)	c.e.
Blood expelled in pial arteries	e.a.
Blood intermittently expelled in pial arteries	i.e.a.
Blood expelled in pial veins	e.v.
Blood intermittently expelled in pial veins	i.e.v.

A figure in the column 'application time' without the above information in brackets means that the blood was expelled from the pial arteries and veins during the whole application time.

Table 33. Normal body temperature. Injection of saline of room temperature into left common carotid artery. Injection pressure 200 mm Hg. Trpb duration 15 min.

Rabbit No	Body wt kg	Body temp. at inj. °C	Saline vol. ml	Inj. time sec	Appl. time sec	Trpb inf. min.	Blood pressure mm Hg		
							Before inj.	At removal of jugular clamps	Rise during inj. from pre-inj. level
C 99	2.2	37.6	33	21	20	6	25	25	2
C 98	2.5	38.4	50	18	16	8	105	70	—4
C 100	2.4	38.2	90 ¹	32	7	5	70	60	24
C 97 ²	2.4	38.0	15	20	0	6	90	75	—10
Mean	2.4	38.1	47	22.8		6	73	58	3
									41

¹ Change of syringes during injection² Injection pressure 300 mm Hg

Table 34. Normal body temperature. Injection of saline of 38° C into left common carotid artery. Injection pressure 200 mm Hg. Trpb duration 15 min.

Rabbit No	Body wt kg	Body temp. at inj. °C	Saline vol. ml	Inj. time sec	Appl. time sec	Trpb inf. min.	Blood pressure mm Hg		
							Before inj.	At removal of jugular clamps	Rise during inj. from pre-inj. level
C 95	2.1	37.8	50	21	20 (i.e.v.)	6 ¹	65	40	14
C 93	2.1	38.2	50	21	13	4	60	50	4
C 94	2.5	38.4	50	18	7	6	60	55	10
C 96	2.8	38.4	50	18	4	4	85	60	8
B 96	1.6	38.4	45	23	20	4	60	—	—
Mean	2.2	38.2	49	20.2		5	66	51	9
									26

¹ Died during trpb inf.

Table 35. Normal body temperature. Injection of saline of 38° C into left common carotid artery with ligation of external carotid artery. Injection pressure 200 mm Hg. Trpb duration 15 minutes.

Rabbit No	Body wt kg	Body temp. at inj. °C	Saline vol. ml	Inj. time sec	Application time sec	Time for re-fill. of pial vessels with blood after end of inj. sec	Trpb inf. min.	Blood pressure mm Hg			
								Before inj.	At removal of jugular clamps	Rise during inj. from pre-inj. level	End of exper.
C 173	2.5	38.2	7	22	20	2	5	90	80	4	90
C 259	1.9	38.0	30	42	40 (c.e. 25 sec, i.e.v. 15 sec)	2	9	90	90	6	45
C 261 ¹	1.8	37.4	9	42	40	4	33 ²	90	80	24	45
C 263 ³	2.0	38.2	10	42	40 (c.e. 34 sec, i.e.v. 6 sec)	1.5	10	95	60	36	40
Mean	2.1	38.0				2.4		91	78	18	55

Head turned to the left during inj. in C 259, 261 and 263.

¹ Injection pressure 240 mm Hg.

² The infusion rate of trpb slow due to narrow catheter.

³ Injection pressure increased to 280 mm Hg.

Table 37. Hypothermia (25° C). Injection of saline of room temperature into left common carotid artery. Injection pressure 200 mm Hg.

Rabbit No	Body temp. at inj. °C	Brain temp. before inj. °C	Saline vol. ml	Inj. time sec	Application time sec	Trpb inf. min.	Trpb dur. min.	Pulse rate per min.		Blood pressure mm Hg		Resp. rate per min.
								Before inj.	End of exper.	Before inj.	End of exper.	
C 29	23.8	24.3	40	24	20	15	15 ¹	104	—	60	—	26—32
C 30	25.0		50	20	16 (c.e. 6 sec, e.a. 10 sec)	12	22 ²	116	0	70	—	28—36
C 31	23.6		40	22	20	17	27	84	0	20	<10	48—58
C 32	23.4	23.8	35	21	20	5	30	80	68	30	<10	48—56
C 33 ³	24.0	24.3	40	24	20	7	30	76	76	20	60	48—56
Mean	24.0	24.1	41	22.2		11		92		40	23	

¹ Breathing pump stopped owing to melt fuse.

² Pulmonary edema 14 minutes after start of trpb infusion.

³ Injection pressure temporarily increased to 220 mm Hg.

Table 36. *Hypothermia (25° C). Cooling data for the experiments presented in table 37.*

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 29	1.7	38.1	26.0	55	93
C 30	1.8	37.8	—	40	75
C 31	1.6	38.0	25.0	40	82
C 32	1.6	38.9	—	35	81
C 33	1.5	38.5	26.2	30	67
Mean	1.6	38.3		40	80

Table 38. *Hypothermia (20° C). Cooling data for the experiments presented in table 39.*

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 60	1.9	37.8	22.4	66	142 ¹
C 63	3.2	38.7	22.6	100	135
C 272	1.9	37.3	21.4	66	103
C 274	1.7	37.2	21.6	56	104
C 276	1.7	37.4	20.2	65	106
C 288	2.0	37.6	21.0	55	89
C 290	1.7	38.2	21.0	45	89
Mean	2.0	37.7	21.5	65	110

¹ Prolonged time for preparation due to technical difficulties.

Table 39. *Hypothermia (26° C). Injection of saline of room temperature into left common carotid artery. Injection pressure 200 mm Hg.*

Rabbit No	Body temp. at inj. °C	Saline vol. ml	Inj. time sec	Application time sec	Time for re-fill. of pial vessels with blood after end of inj. sec	Trpb inf. min.	Trpb dur. min.	Pulse rate per min.			Blood pressure mm Hg		
								Before inj.	End of exper.	Before inj.	Rise during inj. from pre-inj. level	End of exper.	End of exper.
C 60	20.4	42	22	20 (c.e. 17 sec, e.a. 3 sec)	—	20	20—26	52	0	10	—	<10	<10
C 63	21.0	50	25	20 (c.e. 5 sec, e.a. 15 sec)	—	6	8—18	40	0	10	—	<10	<10
C 272 ³	20.8	38	22.5	20	5	22	22—30	56	0	35	6	20	20
C 274 ³	20.4	40	23	20	3.5	24	24—30	52	0	40	10	15	15
C 276 ³	20.4	100 ¹	50.5	31 ²	7	27	30	20	56	30	10	50	50
C 288 ³	20.2	40	23	20	5	18	30	60	68	25	0	25	25
C 290 ³	21.6	40	21.5	20	3	14	5—30	68	0	35	2	<10	<10
Mean	20.7				4.7	19		50		26	6		18

The first figure in the column trpb dur. denotes the point of the last recorded heart-beats.

¹ Change of syringes.

² Total application time. Blood in pial vessels returned during change of syringes.

³ Head turned to the left during inj.

Table 40. Hypothermia (20° C). Cooling data for the experiments presented in table 41.

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 260	1.8	37.2	21.0	66	150 ¹
C 262	1.7	38.2	21.2	70	119
C 264	1.8	37.5	21.0	52	112
C 266	2.3	37.8	21.0	75	118
Mean	1.9	37.7	21.1	66	125

¹ Time for preparation prolonged due to technical difficulties.

Table 41. Hypothermia (20 °C). Injection of saline of room temperature into left common carotid artery with ligation of external carotid artery. Head turned to the left during inj. Injection pressure 200 mm Hg.

Rabbit No	Body temp. at inj. °C	Saline vol. ml	Inj. time sec	Appl. time sec	Time for refill. of pial vessels with blood after end of inj. sec	Trpb dur. min.	Pulse rate per min.		Blood pressure mm Hg		
							Before inj.	End of exper.	Before inj.	Rise during inj. from pre-inj. level	End of exper.
C 260	20.8	75	151.5	150	42	22	30	56	10	2	10
C 262	20.0	130	151.5	150	32.5	15	30	0	20	0	<10
C 264	21.0	140	157	144	5	11 ²	30	64	40	34	20
C 266	20.0	55	152	150	20	17	17—30	52	25	0	25
Mean	20.5	100	153		24.9	16		43	24	9	15

The first figure in the column trpb dur. denotes the point of the last recorded heart-beats. In case of no refilling of the pial vessels with blood during change of syringes this time was included in the application time.

¹ Contractions of heart on opening of chest.² The inferior vena cava cannulated after injection and infusion of trpb started 11 minutes after injection.

Table 42. Normal body temperature. Injection of saline of room temperature into left common carotid artery. Head turned to the left during injection. Injection pressure 200 mm Hg. Trpb duration 15 minutes. No rinsing.

Rabbit No	Body wt kg	Body temp. at inj. °C	Saline vol. ml	Inj. time sec	Appl. time sec	Trpb inf. min.	Blood pressure mm Hg			Macroscopic appearance	Microscopic examination	
							Before inj.	At removal of jugular clamps	Rise during inj. from pre-inj. level		Parenchymal staining	Staining of wall of intracerebral vessels
C 317	1.9	38.4	30	21.5	9.5	6	110	110	4	greyish blue tinge	neg ¹	exceptional staining
C 318	2.0	38.4	30	21	9	4	100	80	16	"	neg	"
C 319	2.1	38.4	40	21	13	5	95	95	30	"	neg	"
Mean	2.0	38.4	33	21.2		5	102	95	17			

¹ Faint, single staining in cerebellum.

Table 43. *Hypothermia (25° C). Cooling data for the experiments presented in table 44.*

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
B 83	3.0	38.6	—	—	82
B 85	2.0	40.2	24.0	62	80
Mean	2.5	39.4			81

Table 44. *Hypothermia (25° C). Infusion of trpb. No rinsing.*

Rabbit No	Body temp. before inf. °C	Trpb inf. min.	Trpb dur. min.	Pulse rate per min.		Macroscopic appearance	Microscopic examination	
				Before inj.	End of exper.		Parenchymal staining	Staining of wall of intracerebral vessels
B 83	25.6	15	25	108	92	greyish blue tinge	neg	neg ¹
B 85	23.0	22	22	88	84	" " "	neg	neg

¹ One vessel stained.

Table 45. *Hypothermia (20° C). Cooling data for the experiments presented in table 46.*

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 324	1.7	38.0	21.0	57	100
C 325	1.9	38.8	21.0	60	100
C 326	1.9	38.6	20.4	62	90
Mean	1.8	38.5	20.8	60	97

Table 46. *Hypothermia (20° C). Injection of saline of room temperature into left common carotid*

Rabbit No	Body temp. at inj. °C	Saline vol. ml	Inj. time sec	Application time sec	Time for re-fill. of pial vessels with blood after end of inj. sec	Trpb inf. min.	Trpb dur. min.
C 324	21.0	42	22	20	3	12	20—30
C 325	20.4	50	22.5	20 (c.e. 12.5 sec, i.e.v. 7.5 sec)	3.5	7	30
C 326	20.2	40	22	20	6	7	15—30
Mean	20.5	44	22.2		4.2	9	

The first figure in the column trpb dur. denotes the point of the last recorded heart-beats.

¹ Single parenchymal staining in septum pellucidum.

² Scattered parenchymal staining within small area in quadrigeminal bodies. Faint staining around 3 vessels in cerebellum and medulla oblongata.

Table 47. *Normal body temperature. Injection of saline of room temperature into left common 15 min. Rinsing*

Rabbit No	Body wt kg	Body temp. at inj. °C	Saline vol. ml	Inj. time sec	Appl. time sec	Trpb inf. min.
C 320	2.5	38.0	35	23	7	5
C 321	2.3	38.6	30	22	10	6
C 322	2.0	38.2	40	23	7	4
Mean	2.3	38.3	35	22.7		5

¹ On the surface defective rinsing within area of middle cerebral artery.

artery. Head turned to the left during injection. Injection pressure 200 mm Hg. No rinsing.

Pulse rate per min		Blood pressure mm Hg			Macroscopic appearance	Microscopic examination	
Before inj.	End of exper.	Before inj.	Rise during inj. from pre-inj. level	End of exper.		Paren- chymal staining	Staining of wall of intracerebral vessels
60	0	60	0	15	greyish blue tinge	neg ¹	exceptional staining
56	20	40	2	20	"	neg	"
36	0	25	2	15	"	neg ²	"
51		42	1	17			

carotid artery. Head turned to the left during inj. Injection pressure 200 mm Hg. Trpb duration with formalin.

Blood pressure mm Hg			Macroscopic appearance	Microscopic examination	
Before inj.	At removal of jugular clamps	Rise during inj. from pre-inj. level		Parenchymal staining	Staining of wall of intracerebral vessels
105	95	—2	neg	neg	exceptional staining
100	90	20	neg	neg	"
105	100	12	neg ¹	neg	"
103	95	10			

Table 48. *Hypothermia (20° C). Cooling data for the experiments presented in table 49.*

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 327	1.9	38.2	21.0	64	100
C 328	2.1	37.6	21.0	65	92
C 329	2.3	37.8	21.0	85	116
Mean	2.1	37.9	21.0	71	103

Table 49. *Hypothermia (20° C). Injection of saline of room temperature into left common carotid*

Rabbit No	Body temp. at inj. °C	Saline vol. ml	Inj. time sec	Appl. time sec	Time for re-fill. of pial vessels with blood after end of inj. sec	Trpb inf. min.	Trpb dur. min.	Pulse rate per min.	
								Before inj.	End of exper.
C 327	20.6	36	22	20	2.5	6	23—30	60	0
C 328	21.2	45	21.5	20	5.5	7	12—30	68	0
C 329	20.4	30	21	20	5.5	10	13—30	56	0
Mean	20.7	37	21.5		4.5	8		61	

The first figure in the column trpb duration denotes the point of the last recorded heart-beats.

Table 50. *Normal body temperature. Injection of saline of room temperature and air*

Rabbit No	Body wt kg	Body temp. at inj. °C	Saline vol. ml	Air in tube ml	Inj. time sec	Application time sec	Trpb inf. min.
C 101	1.8	38.4	45	about 0.02	24	10 ¹	6
C 313	2.3	38.2	50	0.2	23	20	6
C 312	2.3	36.8	60	0.5	26	7 ³	11
C 102	2.4	37.8	40	1—2	—	20 (c.e. 18 sec, e.a. 2 sec)	5
C 103	2.2	38.4	50	1—2	25	14 (e.a.) ¹	5
Mean	2.2	37.9	49		24.5		7

Head turned to the left during inj. in C 312 and 313.

¹ Total application time. Intermittent return of blood during injection.

² After end of injection blood pressure rise to 135 mm Hg.

³ Between the 8th and 15th sec of injection.

artery. Head turned to the left during injection. Injection pressure 200 mm Hg. Rinsing with formalin.

Blood pressure mm Hg			Macroscopic appearance	Microscopic examination	
Before inj.	Rise during inj. from pre-inj. level	End of exper.		Paren- chymal staining	Staining of wall of intracerebral vessels
70	6	10	neg greyish blue tinge right hemisphere and cerebellum neg	neg	neg
40	2	<10		neg	pos
30	0	10		neg	neg
47	3	8			

into left common carotid artery. Injection pressure 200 mm Hg. Trpb duration 15 min.

Blood pressure mm Hg			Degree of injury		Microscopic examination	
Before inj.	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections	Paren- chymal staining	Staining of wall of intracerebral vessels
95	8 ²	90	neg	neg	neg	neg
70	6	40	neg	neg	neg	neg
60	40	40	(+)	(+)	neg	pos
75	8	50	neg	neg	neg	pos
80	10	25	neg	(+?)	neg	pos
76	14	49				

Table 51. *Normal body temperature. Injection of saline of 38° C and air into left common carotid 200 mm Hg. Trpb*

Rabbit No	Body wt kg	Body temp. at inj. °C	Saline vol. ml	Air in tube ml	Inj. time sec	Appl. time sec	Time for re-fill. of pial vessels with blood after end of inj. sec	Trpb inf. min.
C 315	2.0	37.4	15	c:a 0.02	46	40	6	6
C 265	1.5	38.2	22	0.1—0.2	41.5	40	3	9
C 267	2.4	37.9	35	1.0	44	40	2	7
Mean	2.0	37.8	24		43.8	40	3.7	7

Table 52. *Hypothermia (20° C). Cooling data for the experiments presented in table 53.*

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 268	1.5	37.2	21.4	58	108
C 270	1.9	37.6	21.8	55	118
Mean	1.7	37.4	21.6	57	113

Table 53. *Hypothermia (20° C). Injection of saline of room temperature and air into left common pressure*

Rabbit No	Body temp. at inj. °C	Saline vol. ml	Air in tube ml	Inj. time sec	Appl. time sec	Time for refill. of pial vessels with blood after end of inj. sec	Trpb inf. min.	Trpb dur. min.
C 268	20.8	8	0.2	42.5	40	2	10	30
C 270	21.0	15	1.0	50	40	2	16	11—30

The first figure in the column trpb dur. denotes the point of the last recorded heart-beats.

artery with ligation of external carotid artery. Head turned to the left during inj. Injection pressure duration 15 min.

Blood pressure mm Hg				Degree of injury		Microscopic examination	
Before inj.	At removal of jugular clamps	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections	Parenchymal staining	Staining of wall of intracerebral vessels
55	50	20	50	(+)	(+)	pos	pos
75	65	20	40	neg	neg	neg	pos
90	75	44	40	+(+)	+	pos	pos
73	63	28	43				

carotid artery with ligation of external carotid artery. Head turned to the left during inj. Injection 200 mm Hg.

Pulse rate per min.		Blood pressure mm Hg			Degree of injury		Microscopic examination	
Before inj.	End of exper.	Before inj.	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections	Parenchymal staining	Staining of wall of intracerebral vessels
68	16	—	—	10	(+)	(+?)	neg	pos
68	0	50	40	15	(+)	(+?)	neg	pos

Table 54. Normal body temperature. Trpb duration 15 minutes. About 60 ml air in rinsing system.

Rabbit No	Body wt kg	Body temp. °C at inj.	Saline vol. ml	Inj. time sec	Appl. time sec	Blood pressure mm Hg			Macroscopic appearance	Microscopic examination	
						Trpb inf. min.	Before inj.	Rise during inj. from pre-inj. level		Parent-chymal staining	Staining of wall of intracerebral vessels
C 323	2.3	38.0	45	22	4	7	110	30	greyish blue tinge bilaterally in area of a. cerebri media and vermis cerebelli	neg	defect rinsing in macr. stained areas
C 330	1.6	37.2	—	—	—	6	90	—	greyish blue tinge in area of left a. pericallosa, upper part of quadrigeminal bodies and cerebellum	neg ¹	defect rinsing in macr. stained areas

¹ Faint parenchymal staining on 2—3 occasions in medulla oblongata.

Table 55. Normal body temperature. Investigation of areas in brain normally permeable to trpb.

Rabbit No	Body wt kg	Body temp. before inf. °C	Trpb inf. min.	Trpb dur. min.	Blood pressure mm Hg			
					Before inf.	During inf.		End of exper.
						max.	min.	
C 303	2.6	38.4	10	15	110	120	65	90
C 305	1.8	37.5	12	15	100	115	90	65
C 307	2.6	38.2	10	15	115	125	70	110
C 306	1.6	37.4	9	30	55	65	45	20
C 308	1.7	38.0	10	30	—	—	—	—
C 309	2.2	38.2	12	30	90	110	50	85
C 302	2.0	37.0	12	60	105	125	80	85
C 304	1.7	37.8	9	60	90	100	80	60
Mean	2.0	37.8	11		95	109	69	74

Table 56. *Hypothermia (20° C). Cooling data for the experiments presented in table 57.*

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 245	2.2	38.5	21.8	70	98
C 250	2.3	38.6	21.6	74	98
C 251	2.2	38.0	21.6	70	100
C 244	2.5	38.4	21.4	100	125
C 246	2.4	37.8	21.8	85	120
C 247	2.5	38.8	21.8	85	110
C 249	2.2	38.2	21.8	100	125
C 252	2.1	38.2	21.2	75	97
Mean	2.3	38.3	21.6	82	109

Table 57. *Hypothermia (20° C). Investigation of areas in brain normally permeable to trpb.*

Rabbit No	Body temp. before inf. °C	Trpb inf. min.	Trpb dur. min.	Pulse rate per min.		Blood pressure mm Hg		Staining of dura
				Before inf.	End of exper.	Before inf.	During inf. max.	
C 245	20.0	8	10—20	48	0	15	20	faint blue
C 250	21.1	13	8—15	60	0	40	45	faint blue
C 251	20.5	15	9—15	60	0	20	50	blue
C 244	20.6	10	30	48	60	25	70	blue
C 246	20.8	10	30	44	52	—	—	blue
C 247	20.7	17	30	33	0 ¹	20	45	blue
C 249	20.2	13	45—50	28	0	15	60	blue
C 252	19.9	45	60	52	60	20	80	pronounced blue
Mean	20.5	16		47		22	53	

The first figure in the column trpb dur. denotes the point of the last recorded heart-beats.

¹ Occasional contractions of heart on opening of chest.

Table 58. Normal body temperature. Injection of Umbradil 50 % of 38° C into left common carotid artery. Injection pressure 200 mm Hg. Application time aimed at: 20 sec. Trpb duration 15 min.

Rabbit No	Body wt kg	Body temp. at inj. °C	Um-bradil vol. ml	Inj. time sec	Application time sec	Trpb inf. min.	Blood pressure mm Hg		Degree of injury	
							Before inj.	End of exper.	Colour photo	Sections
C 68	2.2	37.8	36	23	20 (c.e. 8 sec, i.e.v. 12 sec)	5	45	35	+	+
C 77	2.1	38.0	25	22	19 (c.e. 10 sec, i.e.a. 9 sec)	5	90	45	+	+
C 78	2.5	38.4	28	23	16 (c.e. 7 sec, e.a. 9 sec) ¹	6	80	35	+	+
C 69	2.7	38.0	32	21	12	5	75	45	+	+
C 72	2.1	38.0	25	22	12 (c.e. 6 sec, e.a. 6 sec)	5	70	50	+	+
C 72	2.6	38.2	25	22	10 (e.a.)	5	90	30	+	+
C 73	2.1	37.9	38	22	10	6	55	25	+	+
C 76	2.5	38.6	27	23	9 (c.e. 4 sec, e.a. 3 sec, i.e.v. 2 sec)	5	70	35	+	+
C 71	2.5	38.4	25	22	6	5	60	25	+	+
C 75	2.2	38.4	40	24	6 (c.e. 2 sec, e.a. 4 sec)	8	40	30	+	+
Mean	2.4	38.2	30	22.4		6	68	36		

¹ Blood returned in pial vessels between the 12th and 16th sec of inj.

Table 59. Normal body temperature. Injection of Umbradil 50 % of room temperature into left common carotid artery. Injection pressure 200 mm Hg. Application time aimed at: 20 seconds. Trpb duration 15 minutes.

Rabbit No	Body wt kg	Body temp. at inj. °C	Umbradil vol. ml	Inj. time sec	Application time sec	Trpb inf. min.	Blood pressure mm Hg			Degree of injury	
							Before inj.	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections
C 74 ¹	2.4	39.0	38	23	20	5	55	—	25	++	++
C 80	2.3	38.0	20	21	20 (c.e. 19 sec, e.a. 1 sec)	5	50	—	35	++ (+)	++
C 81	2.4	39.0	40	22	20	5	50	—	15	++	++
C 84	2.8	38.2	32	23	20	4	70	—	20	++	++
C 85	2.4	37.6	27	22	20 (c.e. 8 sec, i.e.v. 12 sec)	6	95	24	25	++	++
C 87	2.3	38.0	38	22	20	4	80	34	10	++	++
C 88	2.4	38.0	32	23	20 (c.e. 18 sec, e.a. 2 sec)	5	70	40	20	++	++
C 89	2.0	38.0	34	23	20 (c.e. 7 sec, i.e.v. 13 sec)	8	70	32	35	++	++
C 91	1.8	38.2	26	23	20	4	70	42	25	++	++
C 92	2.3	38.4	32	22	20 (c.e. 16 sec, e.a. 4 sec)	4	55	68	25	++	++
C 90	2.1	38.4	28	22	12 (c.e. 3 sec, e.a. 9 sec)	5	70	60	15	++	++
C 82	2.5	39.0	40	22	6	5	60	—	25	++	++
C 83 ¹	2.4	38.4	32	23	3	6	100	—	35	++	++
C 86	2.6	38.2	25	23	3	4	70	58	15	neg	(-)
Mean	2.3	38.3	32	22.4		5	69	45	23		

¹ Injection pressure temporarily increased to 220 mm Hg.

Table 60. *Normal body temperature. Injection of Umbradil 50 % of room temperature into left Application time 10 seconds.*

Rabbit No	Body wt kg	Body temp. at inj. °C	Umbradil vol. ml	Inj. time sec	Application time sec	Time for refill. of pial vessels with blood after end of inj. sec
C 269	2.6	36.6	20	14	10 (c.e. 2 sec, i.e.v. 8 sec)	2.5
C 273	1.7	38.0	16	12.5	10	1.5
C 275 ¹	1.7	37.8	16	17	10 (c.e. 8 sec, i.e.v. 2 sec)	2.5
C 277	1.7	37.6	35	19	10 (c.e. 2 sec ² , e.a. 8 sec ³)	3
C 281	1.9	38.2	11	11	10	2
Mean	1.9	37.6	20	14.7		2.3
C 271 ⁴	2.9	38.2	40	32	0	0

¹ Injection pressure kept between 220 and 240 mm Hg.

² Between the 4th and 6th sec of inj.

³ Between the 11th and 19th sec of inj.

⁴ Injection pressure temporarily increased to 220 mm Hg.

Table 61. *Normal body temperature. Injection of Umbradil 50 % of room temperature into mm Hg. Application time 5*

Rabbit No	Body wt kg	Body temp. at inj. °C	Umbradil vol. ml	Inj. time sec	Application time sec	Time for re-fill. of pial vessels with blood after end of inj. sec
C 283	2.1	38.0	12	7	5	1.5
C 285 ¹	1.9	38.2	15	9	5	2.5
C 287 ¹	1.9	38.0	13	8	5	2.5
C 289	2.1	37.8	15	7.5	5	3
C 292	1.9	37.2	16	7.5	5	3
Mean	2.0	37.8	14	7.8		2.5

¹ Injection pressure temporarily increased to 220 mm Hg.

common carotid artery. Head turned to the left during injection. Injection pressure 200 mm Hg. Trpb duration 15 minutes.

Trpb inf. min.	Blood pressure mm Hg				Degree of injury	
	Before inj.	At removal of jugular clamps	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections
9	50	30	84	30	+++	++
10	105	100	4	30	+++	+++
9	110	105	8	40	+++	+++
9	95	95	34	35	++++	++++
14	100	90	10	70	(+)	(+)
10	92	84	28	41		
2	140	120	40	95	neg	neg

left common carotid artery. Head turned to the left during injection. Injection pressure 200 seconds. Trpb duration 15 minutes.

Trpb inf. min.	Blood pressure mm Hg				Degree of injury	
	Before inj.	At removal of jugular clamps	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections
8	100	100	22	100	+++	+++
6	70	85	44	30	+++	+++
8	90	70	20	70	+++	+++
9	95	65	30	70	+(+)	++
6	80	70	36	30	+++	++
7	90	75	30	60		

Table 62. Normal body temperature. Injection of Umbradil 50 % of 38° C into left common carotid seconds. Trpb

Rabbit No	Body wt kg	Body temp. at inj. °C	Umbradil vol. ml	Inj. time sec	Application time sec	Time for refill. of pial vessels with blood after end of inj. sec
C 186	2.0	37.4	10	42	40 (c.e. 38 sec, e.a. 2 sec)	7
C 188	2.1	38.2	8	44 5	40	8
C 190	1.7	38.0	10	42.5	40	6
C 192	1.7	37.8	11	42	40	4
C 194	1.5	36.2	6	44	40 ¹	2
Mean	1.8	37.5	9	43.0		5.4

¹ Blood in vessels in anterior part of burr hole not expelled.

Table 63. Normal body temperature. Injection of Umbradil 50 % of 38° C into left common carotid 20 seconds.

Rabbit No	Body wt kg	Body temp. at inj. °C	Umbradil vol. ml	Inj. time sec	Application time sec	Time for re-fill. of pial vessels with blood after end of inj. sec
C 171	1.6	38.0	5	22	20	4
C 175	1.4	37.8	5	21.5	20	2
C 180	1.9	37.8	4	21.5	20	3
Mean	1.6	37.9	5	21.6		3

¹ 45 mm Hg immediately after inj.

Table 64. Normal body temperature. Blood pressure reduction by hexamethonium bromide 4 mg/kg pressure 200 mm Hg. Application

Rabbit No	Body wt kg	Body temp. at inj. °C	Umbradil vol. ml	Inj. time sec	Application time sec	Trpb inf. min.
C 106	2.4	38.2	36	23	20	4
C 107	2.2	38.6	30	22	20 (c.e. 15 sec, e.a. 5 sec)	6
C 108	2.3	38.4	28	21	20	7
C 109 ¹	1.6	37.6	37	21	12 (c.e. 5 sec, ² i.e.v. 7 sec ³)	4
C 111	1.9	38.4	25	22	20	6
Mean	2.1	38.2	31	21.8		5

¹ Injection pressure temporarily increased to 250 mm Hg.

² Between the 4th and 9th sec of injection.

³ Between the 14th and 21th sec of injection.

artery with ligation of external carotid artery. Injection pressure 200 mm Hg. Application time 40 duration 15 minutes.

Trpb inf. min.	Blood pressure mm Hg				Degree of injury	
	Before inj.	At removal of jugular clamps	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections
4	95	55	20	30	+++	++++
6	100	95	0	25	++++	++++
4	—	—	—	—	++(+)	++
6	100	60	20	30	++++	++++
2	105	105	—8	30	++++	++++
4	100	79	8	29		

carotid artery with ligation of external carotid artery. Injection pressure 200 mm Hg. Application time 40 duration 15 minutes.

Trpb inf. min.	Blood pressure mm Hg				Degree of injury	
	Before inj.	At removal of jugular clamps	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections
3	95	110	5 ¹	20	++++	++++
6	80	70	10	30	++++	++++
4	85	60	0	60	++++	++++
4	87	80	5	37		

body wt. Injection of Umbradil 50 % of room temperature into left common carotid artery. Injection time 20 seconds. Trpb duration 15 min.

Blood pressure mm Hg						Degree of injury	
Before hex. brom.	After hex. brom.	Before inj.	At removal of jugular clamps	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections
70	35	40	45	5	20	+++	++
85	45	50	55	10	20	+++	+++
60	30	30	30	30	35	+++	+++
80	25	25	25	10	25	+++	+++
110	50	50	50	15	40	+++	+++
81	37	39	41	14	28		

Table 65. Normal body temperature. Injection of Umbradil 50 % cooled in water of about 12° C into left common carotid artery. Head turned to the left during injection. Injection pressure 200 mm Hg. Application time 20 seconds. Trpb duration 15 minutes.

Rabbit No	Body wt kg	Body temp. at inj. °C	Umbradil vol. ml	Inj. time sec	Applica- tion time sec	Time for re- fill. of pial vessels with blood after end of inj. sec	Trpb inf. min.	Blood pressure mm Hg			Degree of injury		
								Before inj.	At removal of jugular clamps	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections
C 293	1.7	36.2	35	22.5	20	12	7	55	60	14	—	+	+
C 294	1.6	37.0	30	24	4 ¹	0	5	70	60	60	40	+	+
C 295	2.0	37.4	32	22	20	4.5	4	55	50	45	30	+	+
C 297	1.7	37.0 ²	30	21.5	20	2.5	9	65	55	25	20	+	+
C 296	1.9	36.2	33	21	20	3.5	8	115	105	40	<10	(+)	(+)
Mean	1.8	36.8	32	22.2		4.5	7	72	66	37	24	+	+

¹ Between the 4th and 8th sec of inj.

² Brain temperature 36.4° C before inj. Fell to a minimum of 35.2° C during injection.

Table 66. *Hypothermia (25° C). Cooling data for the experiments presented in table 67.*

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 23	1.8	38.0	—	40	83
C 24	1.4	38.5	24.4	40	87
C 25	2.3	38.6	27.0	45	85
C 26	1.6	38.6	25.0	30	82
C 27	1.6	38.0	—	40	82
C 28	1.3	37.8	25.6	30	72
C 37	2.0	38.2	27.0	35	81
C 41	2.1	37.0	25.0	50	86
B 34	1.9	38.2	27.0	45	72
B 35	2.0	38.6	28.0	40	75
B 36	1.7	37.4	25.4	45	70
B 37	1.7	38.2	27.6	40	95
B 38	2.7	39.0	27.0	55	95
Mean	1.9	38.2	26.3	41	82

From B 34 to B 38 body temperature recorded on a mercurial thermometer.

Table 67. *Hypothermia (25° C). Injection of Umbradil 50 % of room temperature into*

Rabbit No	Body temp. at inj. °C	Umbradil vol. ml	Inj. time sec	Application time sec	Trpb inf. min.	Trpb dur. min.
C 23	(24.5)	22	24	20	15	30
C 24	24.2	30	23	20	20	30
C 25	25.2	40	23	20	6	30
C 26	24.8	35	23	20	8	30
C 27	(23.4)	30	21.5	20	20	30
C 28	23.2	33	23	20 (c.e. 11 sec, e.a. 9 sec)	4	30
C 37	25.0	40	24	20 (e.a.)	8	30
C 41	24.0	40	25	20 (e.a.)	4	30
B 34	(25.2)	24	26	20	30	20—30
B 35	(27.5)	23	23	20 (c.e. 15 sec, i.e.v. 5 sec)	20	30
B 36	(25.1)	33	22	20	25 ¹	10—25
B 37	(25.3)	26	24	20 (e.a.)	10 ²	10
B 38	(25.6)	33	25	20 (i.e.v.)	10	30
Mean	24.8	31	23.6		14	

The first figure in the column trpb dur. denotes the point of the last recorded heart-beats.

The temperatures given in brackets are interpolated from measurements before and after the injection.

From B 34 to B 38 the rectal temperature was measured on a mercurial thermometer.

¹ 50 ml infused. ² 70 ml infused.

Table 68. *Hypothermia (25° C). Cooling data for the experiments presented in table 69.*

Rabbit No	Body wt kg	Body temperature °C		Lowest temp. after end of cooling	Cooling time min.	
		Before cooling	End of cooling		In water	Until inj.
C 50	2.0	38.2	25.8	24.2	40	193
C 51	1.9	38.5	24.2	23.9	45	175
C 53	3.1	38.4	25.8	23.7	78	182
C 54	2.8	38.2	26.0	24.0	50	180
C 52	3.4	39.0	25.4	24.4	65	192
Mean	2.6	38.5	25.4	24.0	56	184

Table 69. *Hypothermia (25° C). Prolonged time between end of cooling and experiment. Injection of Application time*

Rabbit No	Body temp. at inj. °C	Umbradil vol. ml	Inj. time sec	Application time sec	Trpb inf. min.	Trpb dur. min.
C 50	24.5	38	25	20 (c.e. 14 sec, i.e.v. 6 sec)	14	30
C 51	24.4	35	24	20 (c.e. 10 sec, e.a. 10 sec)	10	26
C 53	24.8	32	22	20	14	30
C 54	25.4	23	22	20	10	30
C 52	24.7	32	25	20 (c.e. 2 sec, i.e.v. 18 sec)	6	6
Mean	24.8	32	23.6		11	

¹ Faint, single parenchymal staining in quadrigeminal bodies.

left common carotid artery. Injection pressure 200 mm Hg. Application time 20 seconds.

Pulse rate per min.		Blood pressure mm Hg		Degree of injury Sections	Microscopic examination	
Before inj.	End of exper.	Before inj.	End of exper.		Parenchymal staining	Staining of wall of intracerebral vessels
100	76	35	20	(+)	pos	pos
96	84	15	<10	neg	neg	pos
104	160	25	20	neg	neg	neg
80	92	45	10	(+)	pos	pos
100	92	40	45	(+)	pos	pos
156	76	85	40	++	pos	pos
132	84	60	20	(+)	pos	pos
70	92	55	20	neg	neg	neg
116	0	—	—	(+)	pos	pos
160	128	—	—	(+)	pos	pos
116	0	—	—	neg	neg	neg
152	0	60	<10	neg	neg	neg
120	96	60	40	(+?)	pos	pos
116		48	23			

Umbradil 50 % of room temperature into left common carotid artery. Injection pressure 200 mm Hg. 20 seconds.

Pulse rate per min.		Blood pressure mm Hg		Degree of injury		Microscopic examination	
Before inj.	End of exper.	Before inj.	End of exper.	Colour photo	Sections	Parenchymal staining	Staining of wall of intracerebral vessels
32	60	10	20	(+)	(+?)	pos	pos
88	0	15	<10	neg	neg ¹	neg ¹	pos
92	64	40	20	++	(+)	pos	pos
92	76	40	40	+	(+)	pos	pos
76	0	20	<10	+(+)	+	pos	pos
76		25	18				

Table 71. *Hypothermia (25° C). Slow cooling. Injection of Umbradil 50 % of room temperature*

Rabbit No	Body temp. at inj. °C	Umbradil vol. ml	Inj. time sec	Application time sec	Trpb inf. min.	Trpb dur. min.
C 42	(23.8)	40	25	20	7	30
C 43	(24.4)	33	23	20 (c.e. 14 sec, e.a. 6 sec)	9	30
C 44	(23.7)	30	24	20	8	30
C 46	(25.4)	25	24	20 (c.e. 10 sec, i.e.v. 10 sec)	18	30
C 47	(25.2)	33	24	20 (c.e. 16 sec, i.e.v. 4 sec)	6	30
C 48	24.6	35	24	20 (c.e. 16 sec, i.e.v. 4 sec)	4	8
Mean	24.5	33	24		9	

The temperatures given in brackets are interpolated from measurements before and after the injection.

¹ Faint, single parenchymal staining in the left cortex.

Table 70. *Hypothermia (25° C). Temperature of cooling water 21—25° C. Cooling data for the experiments presented in table 71.*

Rabbit No	Body wt kg	Body temperature °C		Lowest temp. after end of cooling	Cooling time min.	
		Before cooling	End of cooling		In water	Until inj.
C 42	1.9	37.4	27.0	24.8	60	156
C 43	1.8	36.9	26.4	25.6	120	187
C 44	1.7	37.0	26.8	25.4	105	196
C 46	2.0	37.8	25.6	24.2	120	204
C 47	2.2	38.7	27.6	26.4	120	195
C 48	1.9	38.6	26.6	25.2	90	210
Mean	1.9	37.7	26.7	25.3	103	191

Table 73. *Hypothermia (20° C). Injection of Umbradil 70 % of 38° C into left*

Rabbit No	Body temp. at inj. °C	Umbradil vol. ml	Inj. time sec	Application time sec	Trpb inf. min.	Trpb dur. min.	Pulse per
							Before inj.
C 115	20.8	25	23	20	5	30	64
C 118	21.2	22	22	20 (c.e. 16 sec, e.a. 4 sec)	5	30	72
C 121 ¹	20.6	16	23	20 (c.e. 18 sec, e.a. 2 sec)	9	30	64
C 122	21.8	25	22	20	8	17—25	64
C 123	21.0	28	22	20 (c.e. 17 sec, e.a. 3 sec)	8	30	32
Mean	21.1	23	22.4		7		59

The first figure in the column trpb dur. denotes the point of the last recorded heart-beats.

¹ Injection pressure temporarily increased to 220 mm Hg.

² For a detailed description see text.

into left common carotid artery. Injection pressure 200 mm Hg. Application time 20 seconds.

Pulse rate per min.		Blood pressure mm Hg		Degree of injury		Microscopic examination	
Before inj.	End of exper.	Before inj.	End of exper.	Colour photo	Sections	Parenchymal staining	Staining of wall of intracerebral vessels
108	78	50	20	+(+)	+	pos	pos
100	0	40	10	+	(+)	pos	pos
148	88	70	—	+++	++	pos	pos
96	60	60	—	++	+	pos	pos
88	68	90	30	+	+	pos	pos
100	0	20	—	neg	neg	neg ¹	neg ¹
107		55	20				

Table 72. Hypothermia (20° C). Cooling data for the experiments presented in table 73.

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 115	2.6	38.7	21.0	133 ¹	173
C 118	1.9	38.6	21.0	78	114
C 121	2.2	38.4	21.0	105	144
C 122	2.0	38.2	21.0	100	138
C 123	2.0	38.0	21.6	90	133
Mean	2.1	38.4	21.1	101	140

¹ Shivering during cooling.

common carotid artery. Injection pressure 200 mm Hg. Application time 20 seconds.

rate min.	Blood pressure mm Hg			Degree of injury		Microscopic examination	
End of exper.	Before inj.	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections	Parenchymal staining	Staining of wall of intracerebral vessels
48	35	8	20	(+?)	neg	neg	pos
32	75	10	10	+++	++	pos ²	pos
56	40	2	50	++(+)	(+)	pos ²	pos
0	20	32	<10	+++	(+)	pos ²	pos
32	<10	0	10	+(+)	(+?)	pos ²	pos
	35	10	19				

Table 75. *Hypothermia (20° C). Injection of Umbradil 50 % of room temperature into left*

Rabbit No	Body temp. at inj. °C	Umbradil vol. ml	Inj. time sec	Application time sec	Trpb inf. min.	Trpb dur. min.
C 55	20.9	32	24	20 (c.e. 18 sec, i.e.v. 2 sec)	5	30
C 57	19.7	32	24	20 (c.e. 10 sec, i.e.a. 10 sec)	11	30
C 58	19.0	35	25	20 (i.e.v. 11 sec, i.e.a. 9 sec)	13	30
C 64	20.8	38	24	20 (c.e. 12 sec, i.e.v. 8 sec)	10	30
C 59	20.2	31	23	20 (c.e. 11 sec, i.e.v. 4 sec, i.e.a. 5 sec)	14	14—30
C 61	21.2	33	22	20	6	20—30
C 114	21.4	30	24	20 (c.e. 16 sec, e.a. 4 sec)	8	20—25
C 62	20.6	32	24	20 (c.e. 16 sec, i.e.v. 4 sec)	10 ²	10
Mean	20.5	33	23.8		10	

The first figure in the column trpb dur. denotes the point of the last recorded heart-beats.

¹ Faint, single parenchymal staining in quadrigeminal bodies.

² Tubing to respirator disconnected.

Table 74. *Hypothermia (20° C). Cooling data for the experiments presented in table 75.*

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 55	2.5	38.6	22.0	70	118
C 57	1.7	38.0	21.6	60	100
C 58	2.0	37.5	21.4	80	113
C 64	2.5	39.2	22.6	80	118
C 59	1.9	38.6	22.0	60	97
C 61	2.3	39.4	22.4	72	107
C 114	2.4	37.7	21.0	95	150
C 62	3.0	38.0	22.4	82	118
Mean	2.3	38.4	21.9	75	115

Table 77. *Hypothermia (20° C). Injection of Umbradil 50 % of room temperature into*

Rabbit No	Body temp. at inj. °C	Umbradil vol. ml	Inj. time sec	Application time sec	Time for re-fill. of pial vessels with blood after end of inj. sec	Trpb inf. min.	Trpb dur. min.
C 196	20.4	28	18.5	15	5	8	30
C 198 ¹	20.3	35	20.5	15	3.5	11	14—24
C 200	19.8	27	17	15	31	8	30
C 202	20.6	28	16	15	4	22	40
C 204	20.8	28	22	15 (c.e. 7 sec, e.a. 8 sec)	4	15	60
Mean	20.4	29	18.8		9.5	13	

The first figure in the column trpb dur. denotes the point of the last recorded heart-beats.

¹ Injection into right common carotid artery. Left side intact.

common carotid artery. Injection pressure 200 mm Hg. Application time 20 seconds.

Pulse rate per min.		Blood pressure mm Hg		Degree of injury		Microscopic examination	
Before inj.	End of exper.	Before inj.	End of exper.	Colour photo	Sections	Parenchymal staining	Staining of wall of intracerebral vessels
72	60	30	35	(+?)	(+?)	neg	pos
32	48	10	20	+	(+?)	pos	pos
40	40	10	<10	(+)	(+?)	pos	pos
60	48	20	15	neg	neg	neg	pos
1—2	?	15	—	neg	(+?)	pos	pos
68	0	20	<10	neg	neg	neg ¹	pos
72	0	—	—	neg	(+?)	neg	pos
44	0	25	<10	(+)	(+?)	neg	pos
49		19	14				

Table 76. Hypothermia (20° C). Cooling data for the experiments presented in table 77.

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 196	1.8	37.4	20.6	40	85
C 198	2.0	38.4	21.0	43	85
C 200	1.8	37.6	21.0	35	70
C 202	1.8	38.4	21.0	37	75
C 204	1.8	38.8	21.0	46	87
Mean	1.8	38.1	20.9	40	80

left common carotid artery. Injection pressure 200 mm Hg. Application time 15 seconds.

Pulse rate per min.		Blood pressure mm Hg			Degree of injury		Microscopic examination	
Before inj.	End of exper.	Before inj.	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections	Parenchymal staining	Staining of wall of intracerebral vessels
56	32	55	4	10	(+?)	neg	neg	pos
48	0	45	0	<10	neg	neg	neg	neg
60	16	25	8	<10	neg	neg	neg	pos
56	8	35	5	10	neg	neg	neg	pos
52	20	30	—10	10	neg	neg	neg	pos
54		38	1	8				

Table 79. *Hypothermia (20° C). Injection of Umbradil 50% of 38° C into left*

Rabbit No	Body temp. at inj. °C	Umbradil vol. ml	Inj. time sec	Application time sec	Time for re-fill. of pial vessels with blood after end of inj. sec	Trpb inf. min.	Trpb dur. min.
C 206	20.8	35	23.5	20 (c.e. 11.5 sec, i.e.v. 8.5 sec)	5	8	60
C 208	20.0	30	21.5	20	2	17	60
C 210	20.6	35 ²	22.5	20	6	20	30
C 212	20.0	40	23.5	20	5	6	40
C 214	19.6	40	23	20	5	9	20
Mean	20.2	36	22.8		4.6	12	

¹ Faint, single parenchymal staining in left occipital lobe.² Temperature of Umbradil > 35° C.³ Occasional contractions of heart on opening of chest.Table 78. *Hypothermia (20° C). Cooling data for the experiments presented in table 79.*

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 206	2.1	38.2	21.0	50	96
C 208	1.9	37.8	21.0	43	80
C 210	2.0	38.3	21.0	63	123
C 212	2.2	38.5	21.0	40	88
C 214	1.8	38.7	21.0	45	83
Mean	2.0	38.3	21.0	48	94

Table 81. *Hypothermia (20° C). Treatment with low molecular dextran 6 %. Injection of Umbradil time 20 seconds. Trpb*

Rabbit No	Body temp. at inj. °C	Umbradil vol. ml	Inj. time sec	Application time sec	Trpb inf. min.	Trpb dur. min.	Pulse per
							Before inj.
C 134	20.8	38	24	20 (c.e. 10 sec, e.a. 10 sec)	8	30	72
C 135	20.2	40	23	20	7	30	64
C 136	20.4	40	23	20 (c.e. 19 sec, e.a. 1 sec)	7	30	76
Mean	20.5	39	23.3		7		71

common carotid artery. Injection pressure 200 mm Hg. Application time 20 seconds.

Pulse rate per min.		Blood pressure mm Hg			Degree of injury		Microscopic examination	
Before inj.	End of exper.	Before inj.	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections	Parenchymal staining	Staining of wall of intracerebral vessels
52	44	30	10	50	neg	(+?)	neg ¹	pos
44	44	20	10	20	neg	(+?)	pos	pos
60	0 ³	50	14	<10	neg	neg	neg	pos
48	3	40	14	10	neg	(+?)	pos	pos
20	3	15	16	10	neg	neg	neg	neg
45		31	13	19				

Table 80. Hypothermia (20° C). Treatment with low molecular dextran 6 %. Cooling data for the experiments presented in table 81.

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 134	2.3	37.2	21.0	80	115
C 135	2.3	36.6	21.0	92 ¹	130
C 136	2.3	38.4	21.0	66	117
Mean	2.3	37.4	21.0	79	121

¹ Shivering during cooling.

50 % of room temperature into left common carotid artery. Injection pressure 200 mm Hg. Application duration 30 minutes.

rate min.	Blood pressure mm Hg			Degree of injury		Microscopic examination	
End of exper.	Before inj.	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections	Parenchymal staining	Staining of wall of intracerebral vessels
20	30	6	<10	neg	neg	neg	neg
52	30	8	20	neg	neg	neg	neg
64	50	4	45	(+?)	(+)	pos	pos
45	37	6	23				

Table 83. *Hypothermia (20° C). Treatment with low molecular dextran 10 %. Injection of Umbradil time 20 seconds. Trpb*

Rabbit No	Body temp. at inj. °C	Um-bradil vol. ml	Inj. time sec	Application time sec	Time for re-fill. of pial vessels with blood after end of inj. sec	Trpb inf. min.	Trpb dur. min.
C 149	20.9	—	23	20	9	5	10—11
C 155	20.6	30	25	20	7	7	8—10
C 158	20.5	32	21.5	20 (i.e.v. 20 sec)	15	5	5—18
Mean	20.7	31	23.2		9	7	

The first figure in column trpb dur. denotes the point of the last recorded heart-beats.

Table 82. *Hypothermia (20° C). Treatment with low molecular dextran 10 %. Cooling data for the experiments presented in table 83.*

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 149	2.6	37.0	21.0	95	143
C 155	2.3	36.8	21.0	78	127
C 158	2.0	37.2	20.2	60	110
Mean	2.3	37.0	20.7	78	127

Table 85. *Hypothermia (20° C). Treatment with low molecular dextran 10 %. Injection of Umbradil time 20 seconds. Trpb*

Rabbit No	Body temp. at inj. °C	Um-bradil vol. ml	Inj. time sec	Application time sec	Time for re-fill. of pial vessels with blood after end of inj. sec	Trpb inf. min.	Trpb dur. min.
C 146	20.2	33	23	20	6	9	30
C 147	20.4	28	23	20	11	6	30
C 148	20.0	23	25	20	6	6	30
C 150 ³	20.8	26	23	20 (e.a.)	—	6	30
C 153	20.8	40	23	20	13	6	30
C 162	20.2	30	22	20	4	7	15—22
C 163	20.4	27	22	20 (c.e. 14 sec, e.a. 6 sec)	3	8	30
C 164	20.4	30	23	20	10	5	24
Mean	20.4	30	23		7.6	7	

The first figure in the column trpb dur. denotes the point of the last recorded heart-beats.

¹ Occasional contractions of heart on opening of chest.

² Faint, single parenchymal staining in left cortex.

50 % of room temperature into left common carotid artery. Injection pressure 200 mm Hg. Application duration about 10 minutes.

Pulse rate per min.		Blood pressure mm Hg			Degree of injury		Microscopic examination	
Before inj.	End of exper.	Before inj.	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections	Parenchymal staining	Staining of wall of intracerebral vessels
64	0	40	2	<10	neg	neg	neg	pos
76	0	60	10	<10	neg	neg	neg	pos
60	0	15	2	<10	neg	neg	neg	pos
67		38	5	5				

Table 84. Hypothermia (20° C). Treatment with low molecular dextran 10 %. Cooling data for the experiments presented in table 85.

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 146	2.6	37.4	21.0	113 ¹	159
C 147	2.3	37.4	21.0	80	138
C 148	2.1	36.6	21.0	75	122
C 150	2.6	38.0	21.0	85	158 ²
C 153	2.6	38.4 ³	21.0	95	153
C 162	3.3	38.0	21.0	115	162
C 163	1.8	37.2	21.0	75	123
C 164	2.1	37.2	21.0	64	110
Mean	2.4	37.5	21.0	88	141

¹ Shivering during cooling.

² Prolonged preparation time due to preparation on right side too.

³ Temperature recorded 15—20 min. before start of cooling.

50 % of room temperature into left common carotid artery. Injection pressure 200 mm Hg. Application duration 20—30 minutes.

Pulse rate per min.		Blood pressure mm Hg			Degree of injury		Microscopic examination	
Before inj.	End of exper.	Before inj.	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections	Parenchymal staining	Staining of wall of intracerebral vessels
64	0 ¹	45	2	<10	neg	neg	neg ²	pos
64	52	20	6	15	neg	neg	neg	pos
60	52	40	10	25	neg	neg	neg	pos
56	64	40	0	40	neg	neg	neg	neg
68	64	50	4	20	(+)	(+)	neg ⁴	pos
104	0	40	6	<10	(+)	(+?)	pos	pos
64	2	30	8	<10	(+?)	neg	neg	pos
56	4	40	8	<10	(+)	(+?)	pos	pos
67		38	6	15				

³ Injection into right common carotid artery.

⁴ Faint, single parenchymal staining in quadrigeminal bodies.

Table 87. *Hypothermia (20° C). Treatment with low molecular dextran 10 %. Injection of Umbradil
tion time 20 seconds. Trpb*

Rabbit No	Body temp. at inj. °C	Um-bradil vol. ml	Inj. time sec	Application time sec	Time for re-fill. of pial vessels with blood after end of inj. sec	Trpb inf. min.	Trpb dur. min.
C 151	20.6	30	23	20 (i.e.v.)	6	9	60
C 152	20.6	40	24	20 (i.e.v.)	2	10	55
C 154	20.6	30	22.5	20	—	12	60
C 156	21.0	32	23	20	7	14	60
C 161	21.0	40	25	23 (c.e. 17 sec, i.e.v. 6 sec)	5	18	60
Mean	20.8	34	23.5		5.0	13	

¹ Occasional contractions of heart on opening of chest.Table 86. *Hypothermia (20° C). Treatment with low molecular dextran 10 %. Cooling data for the experiments presented in table 87.*

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 151	2.5	37.2	21.0	80	124
C 152	2.8	36.8	21.0	82	135
C 154	2.1	36.8	21.0	70	120
C 156	2.1	38.2 ¹	21.0	84	136
C 161	1.8	37.0	21.0	55	96
Mean	2.3	37.2	21.0	74	122

¹ Temperature recorded 15—20 min. before start of cooling.Table 89. *Hypothermia (20° C). Treatment with low molecular dextran 10 %. Injection of Umbradil
tion time 20 seconds. Trpb*

Rabbit No	Body temp. at inj. °C	Um-bradil vol. ml	Inj. time sec	Application time sec	Time for re-fill. of pial vessels with blood after end of inj. sec	Trpb inf. min.	Trpb dur. min.
C 166	21.0	38	22	20 (c.e. 10 sec, e.a. 10 sec)	3	8	105
C 157	20.6	35	20	18 ²	0	5	120
C 159	20.6	30	22	20	5	8	120
C 160	20.9	33	21.5	20 (c.e. 18 sec, e.a. 2 sec)	6.5	10	120
C 167	21.0	30	24	20 (e.a.)	3	7	120
Mean	20.8	33	21.9		3.5	8	

¹ Occasional contractions of heart on opening of chest.² Pial vessels refilled with blood during inj.³ Only occasionally.

50 % of room temperature into left common carotid artery. Injection pressure 200 mm Hg. Application 60 minutes.

Pulse rate per min.		Blood pressure mm Hg			Degree of injury		Microscopic examination	
Before inj.	End of exper.	Before inj.	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections	Parenchymal staining	Staining of wall of intracerebral vessels
60	40	50	6	60	(+?)	(+?)	pos	pos
60	0 ¹	65	2	< 10	neg	(+?)	pos	pos
60	40	40	2	10	neg	neg	neg	pos
72	32	60	6	20	(+?)	(+)	pos	pos
68	4	20	4	10	neg	neg	neg	neg
64		47	4	21				

Table 88. Hypothermia (20° C). Treatment with low molecular dextran 10 %. Cooling data for the experiments presented in table 89.

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 166	1.7	38.8	21.0	50	95
C 157	1.9	36.8	21.0	60	115
C 159	1.8	38.6	21.0	55	96
C 160	1.8	37.2	21.0	57	100
C 167	2.0	37.0	21.0	70	114
Mean	1.8	37.7	21.0	58	104

50 % of room temperature into left common carotid artery. Injection pressure 200 mm Hg. Application 105—120 minutes.

Pulse rate per min.		Blood pressure mm Hg			Degree of injury		Microscopic examination	
Before inj.	End of exper.	Before inj.	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections	Parenchymal staining	Staining of wall of intracerebral vessels
64	0 ¹	30	4	< 10	neg	(+?)	pos	pos
60	52	50	4	25	(+)	neg	pos	pos ³
60	44	30	2	< 10	neg	neg	neg	neg
68	28	50	6	10	(+?)	(+?)	pos	pos
64	44	70	6	25	neg	(+?)	pos	pos
63		46	4	14				

Table 91. Hypothermia (20° C). The

Rabbit No	Body temperature at inf. °C	Blood pressure	
		Before inf.	During inf. of saline
C 243	21.8	60	
C 248	20.4	30	40
C 253	19.8	20	25
C 257 ¹	19.4	30	
C 258 ¹	19.8	20	
Mean	20.2	32	

¹ Noradrenaline solved in trpb solution.

Table 90. Hypothermia (20° C). Cooling data for the experiments presented in table 91.

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 243	2.2	38.0	22.0	75	110
C 248	2.2	38.1	21.6	100	138
C 253	1.7	38.0	21.0	77	126
C 257	1.8	37.4	20.9	60	91
C 258	2.1	37.6	20.6	78	114
Mean	2.0	37.8	21.2	78	116

Table 93. Hypothermia (20° C). Blood pressure increase by noradrenaline. Injection of Umbradil pressure 200 mm Hg. Applic-

Rabbit No	Body temp. at inj. °C	Um-bradil vol. ml	Inj. time sec	Application time sec	Time for re-fill. of pial vessels with blood after end of inj. sec	Trpb inf. min.	Trpb dur. min.
C 280	21.2	34	25	20	3.5	22	30
C 282	21.2	35	21	20	1	25	30
C 286	21.4	28	22	20	4	15	30
C 279	20.6	38	26	20	8	6	13—30
C 284	21.0	30	22	20 (c.e. 15 sec, i.e.v. 5 sec)	3.5	28	21—30
Mean	21.1	33	23.2		4.0	19	

The first figure in the column trpb dur. denotes the point of the last recorded heart-beats.

¹ Occasional contractions of heart on opening of chest.² Only exceptionally.

effect of noradrenaline on blood pressure.

mm Hg		Pulse rate per min.		
During noradr.		Before inf.	During noradr.	End of exper.
max.	level			
85	85	72	72	72
95	95	32	42	42
70	70	40	96	80
90	90—75	40	100	100
85	85—40	36	54	56
		44	73	70

Table 92. *Hypothermia (20° C). Cooling data for the experiments presented in table 93.*

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 280	1.8	38.0	21.7	50	125
C 282	2.0	37.7	21.2	65	113
C 286	1.7	37.6	21.0	45	92
C 279	2.1	37.8	21.4	64	97
C 284	2.0	38.2	21.5	85 ¹	127
Mean	1.9	37.9	21.4	62	111

¹ Shivering during first part of cooling.

50 % of room temperature into left common carotid artery. Head turned to the left during inj. Injection time 20 seconds.

Pulse rate per min.		Blood pressure mm Hg				Degree of injury		Microscopic examination	
Be-fore inj.	End of exper.	Be-fore nor-adr. inf.	During noradr. inf. be-fore inj.	Rise du-ring inj. from pre-inj. level	End of exper.	Colour photo	Sections	Paren-chymal staining	Staining of wall of in-tracerebral vessels
20	0 ¹	65	105	0	10	(+?)	(+?)	pos	pos
44	0 ¹	30	90	—8	15	(+?)	neg	neg	pos
60	52	25	55	—2	20	neg	neg	neg	pos ²
32	0	20	60	6	—	neg	neg	neg	pos
48	0	30	70	18	15	(+?)	(+?)	pos	pos
41		34	76	3	15				

Table 95. *Hypothermia (20° C). Injection of Umbradil 70 % of 38° C into left common carotid sure 200*

Rabbit No	Body temp. at inj. °C	Um-bradil vol. ml	Inj. time sec	Application time sec	Time for re-fill. of pial vessels with blood after end of inj. sec	Trpb inf. min.	Trpb dur. min.
C 216	20.0	15	43.5	40	11.5	8	30
C 218	20.6	16.5	42	40	8	13	30
C 220	20.4	8	63.5	60	7	8	30
C 225	20.4	12	99	90	13	6	15
C 223	20.8	40	157	150	30	5	15
Mean	20.4					8	

¹ Occasional contractions of heart on opening of chest.

Table 94. *Hypothermia (20° C). Cooling data for the experiments presented in table 95.*

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 216	2.0	37.8	21.0	45	90
C 218	2.1	38.0	21.0	50	93
C 220	1.6	38.2	21.0	45	88
C 225	1.5	38.0	21.0	55	105
C 223	1.4	38.2	21.0	45	110
Mean	1.7	38.0	21.0	48	97

Table 97. *Hypothermia (20° C). Injection of Umbradil 50 % of room temperature into left common time 40*

Rabbit No	Body temp. at inj. °C	Um-bradil vol. ml	Inj. time sec	Application time sec	Time for re-fill. of pial vessels with blood after end of inj. sec	Trpb inf. min.	Trpb dur. min.
C 185	20.4	—	41	40	4	9	30—38
C 187	20.0	8	42	40	4	7	29
C 191	20.8	12	41.5	40	4	6	29—44
C 193	20.8	8	42.5	40	4	6	11—16
C 189 ¹	20.0	5	42	40 (c.e. 30 sec, e.a. 10 sec)	6	9	12
Mean	20.4	8	41.8		4.4	7	

The first figure in the column trpb dur. denotes the point of the last recorded heart-beats.

¹ Jugular veins intact.

² Occasional contractions of heart on opening of chest.

artery with ligation of external carotid artery. Head turned to the left during injection. Injection pressure 200 mm Hg.

Pulse rate per min.		Blood pressure mm Hg			Degree of injury		Microscopic examination	
Before inj.	End of exper.	Before inj.	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections	Parenchymal staining	Staining of wall of intracerebral vessels
16	20	20	0	10	(+)	(+?)	pos	pos
44	12	45	8	10	+(+)	(+)	pos	pos
56	0 ¹	30	6	10	(+)	(+)	neg	pos
20	0 ¹	20	2	10	+(+)	(+?)	pos	pos
24	0 ¹	10	8	<10	++(+)	++++	pos	pos
32		25						

Table 96. Hypothermia (20° C). Cooling data for the experiments presented in table 97.

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 185	1.7	37.8	21.0	48	85
C 187	1.8	37.4	21.0	50	92
C 191	1.9	37.7	21.6	67	110
C 193	1.7	37.8	21.0	40	91
C 189	1.8	37.6	21.0	55	100
Mean	1.8	37.7	21.1	52	96

carotid artery with ligation of external carotid artery. Injection pressure 200 mm Hg. Application seconds.

Pulse rate per min.		Blood pressure mm Hg			Degree of injury		Microscopic examination	
Before inj.	End of exper.	Before inj.	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections	Parenchymal staining	Staining of wall of intracerebral vessels
48	0	35	6	<10	(+)	(+)	pos	pos
60	0 ²	35	8	<10	neg	(+?)	pos	pos
44	3	10	18	<10	++	+	pos	pos
64	0 ²	35	8	10	+	+	pos	pos
64	3	20	2	<10	neg	neg	neg	pos
56		27	8	6				

Table 98. *Hypothermia (20° C). Cooling data for the experiments presented in table 99.*

Rabbit No	Body wt kg	Body temperature °C		Cooling time min.	
		Before cooling	End of cooling	In water	Until inj.
C 174	1.6	37.7	21.0	50	100
C 177	2.2	38.0	21.0	72	118
C 179	1.7	37.8	21.0	65	118
C 181	1.8	37.8	21.0	50	93
C 183	1.9	38.4	21.0	55	95
Mean	1.8	37.9	21.0	58	105

Table 99. *Hypothermia (20° C). Injection of Umbradil 50 % of room temperature into left common*
20

Rabbit No	Body temp. at inj. °C	Um-bradil vol. ml	Inj. time sec	Application time sec	Time for re-fill. of pial vessels with blood after end of inj. sec	Trpb inf. min.	Trpb dur. min.
C 174 ¹	20.0	4	22.5	20	6	30	35
C 177	20.0	3	25	20	10	10	19
C 179	20.0	4	22	20	14	6	8—16
C 181	20.0	5	21	20	10	5	15—30
C 183	20.0	4	21	20	4	13	18
Mean	20.0	4	22.3		8.8	13	

The first figure in the column trpb dur. denotes the point of the last recorded heart-beats.

¹ Injection pressure temporarily increased to 220 mm Hg.

² Small area stained in left fronto-parietal region.

³ Staining of macr. blue stained area.

⁴ Occasional contractions of heart on opening of chest.

carotid artery with ligation of external carotid artery. Injection pressure 200 mm Hg. Application time seconds.

Pulse rate per min.		Blood pressure mm Hg			Degree of injury		Microscopic examination	
Before inj.	End of exper.	Before inj.	Rise during inj. from pre-inj. level	End of exper.	Colour photo	Sections	Parenchymal staining	Staining of wall of intracerebral vessels
60	48	30	4	80	neg ²	neg ²	neg ³	neg ³
28	0 ⁴	25	—4	<10	neg	neg	neg	pos
36	0	20	—5	<10	neg	neg	neg	pos
52	0	30	2	10	neg	neg	pos	pos
60	0 ⁴	30	0	<10	(+?)	(+?)	pos	pos
47		27	—1	21				

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PLATES

PLATE I

1. Normal body temperature. Injection of Umbradil 50 % of room temperature into left common carotid artery. Application time 20 sec. Trpb duration 15 min. Rated as + + +.
2. Same as in 1. Cut surface. Pronounced injury to BBB rated as + + +.
3. Hypothermia (20° C). Treatment with low molecular dextran 10 % during cooling. Injection of Umbradil 50 % of room temperature into left common carotid artery. Application time 20 sec. Trpb duration 60 min. Rated as (+ ?).
4. Same as in 3. Cut surface. Low-grade injury to BBB rated as (+).



1



2



3



4

PLATE II

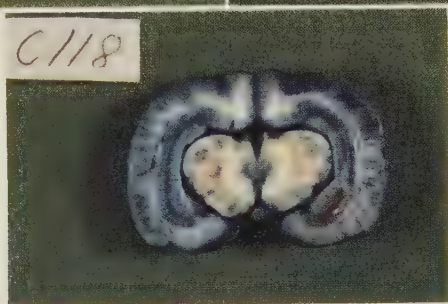
1. Hypothermia (20° C). Injection of Umbradil 70 % of 38° C into left common carotid artery. Application time 20 sec. Trpb duration 30 min. Rated as + + +.
2. Same as in 1. Cut surface. In the basal ganglia on the left side pronounced injury to BBB rated as + + +. Area with stasis in the right hemisphere nearest to the midline.
3. Same as in 1. Cut surface. Note staining in the outer layer of the cortex and the streaky staining with areas of less pronounced staining.
4. Hypothermia (20° C). Injection of Umbradil 70 % of 38° C into left common carotid artery. Application time 20 sec. Trpb duration *in vivo* 17 min. Intense staining of the surface of the brain rated as + + +.
5. Same as in 4. Cut surface. Low-grade injury to BBB rated as (+). Note difference in degree of staining between the surface and the cut surface.



1



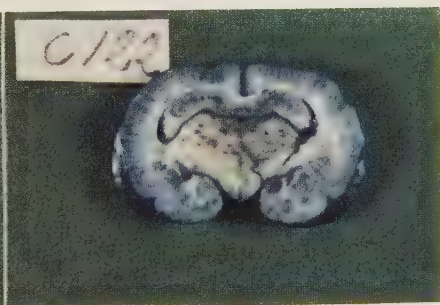
2



3



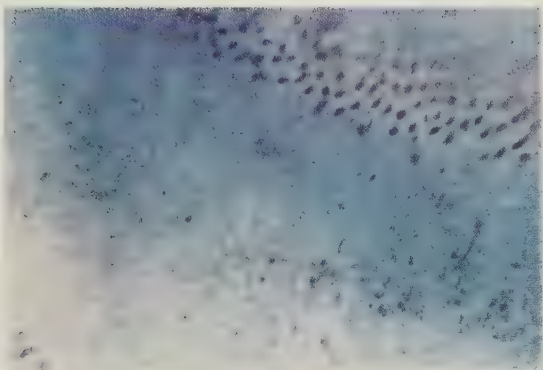
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5

PLATE III

1. Microphotograph. Normal body temperature. Injection of Umbradil 50 % of room temperature into common carotid artery. Application time 20 sec. Trpb duration 15 min. From cerebellum in C 91. Note staining of the nuclei of the Purkinje cells, diffuse staining in granular layer with occasional staining of parenchymal nuclei. The vessels are filled with trpb in spite of the rinsing. $\times 60$.
2. Microphotograph. Hypothermia (20°C). Injection of Umbradil 70 % of 38°C into common carotid artery. Application time 20 sec. Trpb duration *in vivo* 17 min. From left pyriform lobe in C 122. Note pronounced damage to the vessel wall with staining of the endothelial cell nuclei and virtual absence of staining of the parenchyma. Vessels well rinsed. $\times 75$.



1



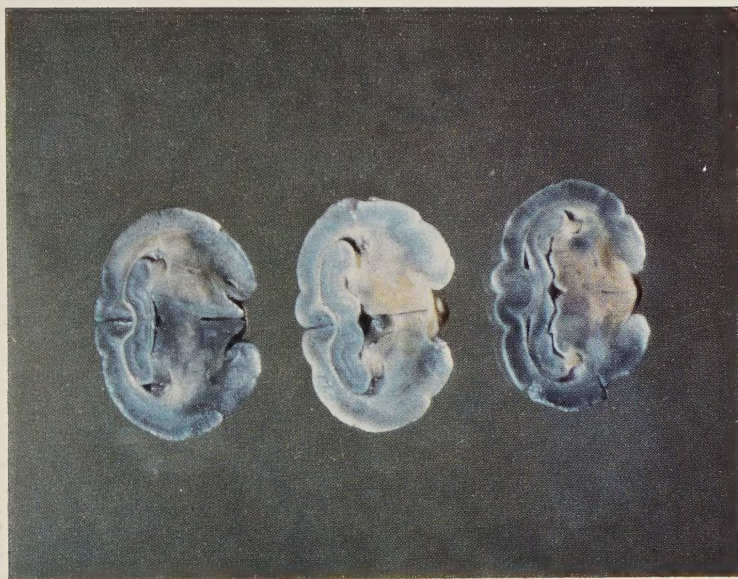
2

PLATE IV

Injection of sodium desoxycholate 0.05 % into common carotid artery. Application time 20 sec.

- a. Normal body temperature. Trpb duration 15 min.
- b. Hypothermia (20° C). Trpb duration 15 min.
- c. Hypothermia (20° C). Trpb duration 30 min.

Note that the injury to BBB in hypothermia is not diminished when the length of trpb duration is doubled as compared with normothermia (From JEPSSON, unpublished data).



a

b

c

